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ZYTOGENETISCHE UNTERSUCHUNGEN AN MISCHLINGEN DER GATTUNG DICRANURA B. (LEPIDOPTERA)

VON *HARRY FEDERLEY*
HELSINGFORS, FINNLAND

I. EINLEITUNG.

SCHON im zweiten Jahrzehnt dieses Jahrhunderts plante der Verfasser inter- und intraspezifische Kreuzungen mit Arten der Lepidopterengattung *Dicranura* anzustellen, vor allem in der Absicht das Verhalten der Chromosomen bei den verschiedenen Mischlingen einer eingehenden Untersuchung zu unterwerfen. Diese Pläne kamen jedoch nur teilweise zur Ausführung. Sie wurden durch den Weltkrieg unterbrochen. Nur über die Resultate der Spezieskreuzung *D. erminea* ♀ × *vinula* ♂ wurde eine kurze Mitteilung veröffentlicht (FEDERLEY, 1915 b). Der Weltkrieg verhinderte nämlich die Anschaffung von neuem Zuchtmaterial, und erst mehrere Jahre nach Abschluss des Krieges gelang es wieder Eier von *Dicranura*-Rassen zu erhalten; es waren solche der in Ostasien vorkommenden Rassen *erminea* v. *menciana* MOORE und *vinula* v. *felina* BUTL. Leider glückte es nicht die Raupen dieser Formen zur Verpuppung zu bringen, und erst Anfang der dreissiger Jahre war es wieder möglich Zuchtmaterial zu bekommen, diesmal von der in Albanien vorkommenden Form von *vinula* — hier *albanica* genannt — und der in Marokko fliegenden Varietät *Delavoiei* GASCHET dieser Art. Die höchst interessanten Chromosomenzahlen dieser Varietäten sowie die eigenartigen Chromosomenverhältnisse der Mischlinge zwischen der marokkanischen und der finnländischen Form habe ich gleichfalls kurz besprochen und abgebildet (FEDERLEY, 1939, 1940). Auch über das Resultat der Kreuzung zwischen der nord- und der mitteleuropäischen Form liegt eine kurze Mitteilung vor (FEDERLEY, 1937). Es war eigentlich meine Absicht die Versuche fortzusetzen und vor allem das Material der in theoretischer Hinsicht so wichtigen Chromosomenverhältnisse zu vervollständigen. Da jedoch mein eigenes Zuchtmaterial infolge vieler unglücklicher Umstände verloren ging und neues während des neuen Weltkrieges unmöglich zu beschaffen ist, habe ich mich dafür entschlossen die er-

zielten Resultate — seien sie auch unvollständig — schon jetzt zu veröffentlichen.

II. DIE EXPERIMENTELLEN RESULTATE.

Die Notodontiden-Gattung *Dicranura* B. ist in der paläarktischen Region von zwei Arten vertreten, *D. erminea* ESP. und *D. vinula* L. Die Art *Przwalski* ALPH. betrachten STAUDINGER und REBEL (1901) bloss als eine *forma Darwiniana* von *erminea*. In Nord-Amerika scheint die Gattung überhaupt nicht vorzukommen. Wenigstens wird sie von PACKARD (1895) in seiner grossen Monographie der Notodontiden Nord-Amerikas nicht erwähnt. PACKARD führt nur fünf Arten der nahe verwandten Gattung *Cerura* SCHRNK. an, welche Arten alle weit kleiner sind und mit den paläarktischen Vertretern dieser Gattung grosse habituelle Ähnlichkeit haben. Eine Kreuzung zwischen den *Dicranura*- und den *Cerura*-Arten dürfte kaum möglich sein, schon wegen des erheblichen Grössenunterschiedes. Alle meine Versuche in dieser Beziehung sind resultatlos geblieben. Allerdings sind sie mit einem verhältnismässig geringen Material ausgeführt worden.

Sowohl *erminea* als *vinula* haben eine sehr weite Verbreitung, von Frankreich im Westen bis China und Japan im Osten, und treten in diesem grossen Gebiete in verschiedenen Varietäten auf. *Vinula* ist ausserdem noch in Nord-Afrika durch die Varietät *Delavoiei* GASCHET vertreten. Es scheint zweifelhaft, ob nicht einige dieser Varietäten eher als selbständige Arten aufzufassen sind, eine Frage auf die wir in anderem Zusammenhang zurückkommen werden.

Die beiden Arten *erminea* und *vinula* lassen sich leicht kreuzen, wenn *erminea* als Weibchen benutzt wird. Die reziproke Kreuzung dürfte dagegen nicht bekannt sein. Die Varietäten der beiden Arten sind intraspezifisch sehr leicht kreuzbar. Ein Umstand, der bei den Kreuzungsversuchen jedoch grosse Schwierigkeiten bietet und mir von sehr grossem Ärgernis gewesen ist, ist das äusserst unregelmässige Ausschlüpfen der Falter aus der Puppe. Nur einige aus Deutschland erhaltene Zuchten von *vinula* — hier *germanica* genannt — ergaben die Falter innerhalb einer ziemlich kurzen Zeit. Die in Finnland fliegende Form *fennica* SCHULTZ schlüpft dagegen von Mitte Mai bis Mitte Juli und ergibt dazu nur einen kleinen Teil der Falter nach einjähriger Puppenruhe. Eine zweimalige Überwinterung der Puppe ist das gewöhnliche, eine drei- und sogar viermalige Überwinterung kommt vor, ist jedoch seltener. Ähnlich verhalten sich auch andere von mir ge-

züchtete Varietäten von *vinula*. Will man Kreuzungen ausführen, muss man also ein sehr grosses Material zu seiner Verfügung haben, denn nur dann kann man damit rechnen eine genügende Anzahl Weibchen und Männchen der zu kreuzenden Varietäten gleichzeitig zu erhalten. Da die Raupen der *Dicranura*-Arten als erwachsen sehr empfindlich sind und leicht zu Grunde gehen — vermutlich durch die Wirkung eines Virus — ist es mit Schwierigkeiten verbunden eine grössere Anzahl Puppen zu erhalten, und man kann die gewünschte Kreuzungsanalyse wegen Mangel an Zuchtmaterial nicht durchführen.

Fennica × *germanica*. — Den ersten Antrieb zu der vorliegenden Untersuchung gab der Wunsch das Resultat dieser Kreuzung kennen zu lernen. Die Fragestellung war zunächst eine rein genetisch-evolutionistische.

Die Männchen der im Norden — Schweden und Finnland — fliegenden *vinula*-Rasse *fennica* haben fast ganz durchsichtige Flügel, so dass nur der proximale Teil der Vorderflügel Zeichnungselemente aufweist, wogegen der distale Teil ganz durchsichtig und zeichnungslos ist. Die Durchsichtigkeit wird teils durch Verringerung der Schuppenzahl, teils durch Verkleinerung der Schuppengrösse hervorgerufen (FEDERLEY, 1910). Die Männchen der auf dem europäischen Kontinent vorkommenden Form sind dagegen normal beschuppt und den Weibchen in dieser Beziehung ganz ähnlich. In Dänemark scheinen beide Typen von Männchen vorzukommen. Auch in den baltischen Ländern wird die durchsichtige Form dann und wann gefunden. Die Weibchen von *fennica* können von denjenigen von *germanica* nicht unterschieden werden.

Es schien mir eine verlockende Aufgabe zu sein durch Kreuzung der beiden Formen das genetische Verhalten derselben zueinander zu ergründen. War die eine Form durch eine einfache Mutation aus der anderen entstanden oder lag ein Unterschied von komplizierterer Art vor? Gleichzeitig war es meine Absicht das Verhalten der Chromosomen des Mischlings zum Gegenstand meiner Untersuchung zu machen. Es hatte sich nämlich gezeigt, dass auch Mischlinge zwischen nahe verwandten aber in geographischer Hinsicht gut isolierten Formen einer Art starke Störungen in der Spermatogenese aufweisen (FEDERLEY, 1914, 1915 a).

Die reziproken Kreuzungen zwischen *fennica* und *germanica* ergaben in mehreren Versuchen, dass das Mischlingsmännchen einen intermediären Typus vertritt. Der durchsichtige Teil des Vorderflügels ist kleiner als bei *fennica* und die Andeutungen der Zeichnungselemente treten deutlicher hervor.

Die Rückkreuzungen und die F_2-Generation konnten die Analyse nicht weiter führen. Leider standen mir nur ganz wenige Exemplare zur Verfügung, aber alle reden dieselbe Sprache. Die Rückkreuzungsmännchen bilden wieder intermediäre Typen zwischen dem F_1-Elter und der benutzten Ausgangsrasse, und die wenigen F_2-Männchen gleichen ganz den F_1-Männchen, zeigen also keine Zeichen einer Spaltung. Es ist demnach klar, dass es sich nicht um eine einfache Mutation handelt, sondern dass die beiden Formen durch tiefer eingreifende Unterschiede von einander getrennt sind. Unter solchen Umständen schien es mir nicht der Mühe wert die Analyse fortzusetzen, erstens weil die Raupen grosse Sterblichkeit aufwiesen, und zweitens weil das studierte Merkmal für eine eingehende Analyse mir wenig geeignet erschien.

Arctica ♀ × *vinula* ♂. — Im hohen Norden tritt *vinula* in mehr oder weniger melanistischen Formen auf. Bei der Form *phantoma* DALM. sind sowohl Flügel als Körper fast ganz schwarz gefärbt, bei der Form *arctica* ZETT. sind dagegen nur die schwarzen Zeichnungselemente stärker ausgeprägt und der Grundton der Flügel dunkler.

Die Kreuzung *arctica* ♀ × *vinula* ♂ wurde im Sommer 1933 von Herrn K. JOHN in Altenburg, Sachsen, zwischen einem finnländischen *arctica* ♀ und einem deutschen *vinula* ♂ erzielt. Aus dieser Kreuzung sandte mir Herr JOHN ein Dutzend Puppen, die jedoch nur ein ♂ ergaben; die übrigen Puppen waren vertrocknet. Dieses einzige ♂ war ein wenig dunkler als die *fennica* ♂♂ und zeigte an dem Aussenrande der Vorderflügel Andeutungen der Zeichnung, die jedoch weniger deutlich waren als diejenigen der reinen *germanica* ♂♂. Es war also im grossen und ganzen dem F_1-Männchen der Kreuzung *fennica* × *germanica* recht ähnlich.

Im folgenden Sommer 1934 erhielt ich von Herrn JOHN 30 Eier, die aus einer Kopula der F_1-Tiere dieser Kreuzung stammten. Auch diese Zucht misslang, indem ich nur 6 Puppen erhielt, von denen 5 Imagines ergaben. Von diesen waren 3 ♂♂, die hell waren und wie das F_1-Männchen aussahen; die beiden ♀♀ waren nur unbedeutend dunkler als die typischen *fennica* ♀♀.

Delavoiei ♀ × *fennica* ♂. — Die afrikanische Form *Delavoiei* GASCHET unterscheidet sich von der Hauptform *germanica* dadurch, dass die Flügel beider Geschlechter einen eigenartigen schmutzig gelbgrauen Farbenton haben, der ganz besonders an den Hinterflügeln, die bei dem Männchen der Hauptform schneeweiss sind, auffällt. Die Zeichnung ist dieselbe wie diejenige der Hauptform, aber rein schwarze

Elemente wie bei der Hauptform kommen weder an den Flügeln noch an dem Körper vor.

Auch die Eier sind verschieden. Während sie bei allen von mir untersuchten Formen von *vinula* einfarbig lederfarben bis dunkel rotbraun sind, tragen die Eier von *Delavoiei* immer an der Basis einen weissen Rand.

Die Raupen von *Delavoiei* können auch meistens von den typischen *vinula*-Raupen unterschieden werden. Die neuausgeschlüpften Raupen zeigen in der Regel einen eigenartigen Dimorphismus. Neben der typischen dunkel purpurfarbenen Raupe tritt eine hellere auf, die eine hellbraune terrakottafarbene Dorsalbinde hat. Die hellen Raupen waren immer in der Minderzahl. In einer Zucht war das Verhältnis 77 hellbraune : 95 purpurfarbene, in einer anderen Zucht war ein Drittel der Raupen hell. Nur in einer einzigen Zucht waren alle Raupen hell, was den Verdacht erweckt, dass es sich um eine rezessive Eigenschaft handelt. Auch die älteren Raupen zeigen deutliche Unterschiede von der typischen *vinula*-Raupe. Der für diese charakteristische Dorsalfleck ist nicht braunlila oder hellblau, sondern von einer sehr eigenartigen graugrünen Farbe und von einem kanariengelben, nicht weissen Rand, wie bei der Hauptform, umgeben. Auch in der Form ist der Fleck verschieden. Er ist bei *Delavoiei* viel schmäler und läuft nicht auf den Körpersegmenten 8—9 nach unten aus, wie dies bei *vinula* die Regel ist.

Allem nach zu urteilen handelt es sich hier eher um eine Art als um eine Varietät, was die Untersuchung der Chromosomenverhältnisse sowohl bei *Delavoici* als bei dem Mischling *Delavoiei* ♀ × *fennica* ♂ bestätigt hat.

Die F_1-Individuen sind im grossen und ganzen intermediär, stehen jedoch *fennica* näher. Die neuausgeschlüpfte Raupe ist immer dunkel purpurfarben, die ältere auch *fennica* ähnlicher. Von vielen Zuchten ergab nur eine Puppen, 10 ♂♂ und nur 3 ♀♀. Leider war es mir unmöglich eine F_2-Generation zu erhalten. Die Puppen lagen nämlich 2—4 Jahre, keine einzige ergab den Falter nach der ersten Überwinterung. Im zweiten und dritten Sommer schlüpften lauter Männchen aus, und in dem vierten zuerst die Weibchen, die Männchen erst, als die Weibchen schon so alt waren, dass keine Kopula eingegangen wurde. Um zu zeigen mit welchen Schwierigkeiten eine Kreuzungsanalyse in der Gattung *Dicranura* verbunden ist, gebe ich hier die Zeiten des Ausschlüpfens dieses Mischlings wieder. Die Daten reden eine deutlichere Sprache als alle Auseinandersetzungen und beweisen klar die Schwierigkeiten hier Kreuzungen zu erzielen.

18. V. 1938	.....	1 ♂
22—26. V. 1939	.....	5 ♂♂
14—19. VI. 1940	.....	3 ♀♀
23—24. VI. »	.....	2 ♂♂

Wie schon hervorgehoben wurde, handelt es sich wahrscheinlich um eine Spezies-, nicht um eine Rassenkreuzung, weshalb eine fortgesetzte Kreuzungsanalyse kaum irgendwelche Aufschlüsse über die genetischen Beziehungen der beiden Arten zu einander ergeben hätte. Dagegen wären die F_2-Individuen in zytologischer Hinsicht von allergrösstem Interesse gewesen.

Albanica ♀ × *germanica* ♂. — Durch Herrn KURT JOHN erhielt ich eine Anzahl Eier, die von einem aus Albanien stammenden Weibchen abgelegt und von einem albanischen Männchen befruchtet waren. Sowohl Eier wie Raupen zeigten grosse Ähnlichkeit mit denjenigen der typischen *vinula*. Die Falter unterscheiden sich dagegen deutlich von der letztgenannten und können als einen intermediären Typus zwischen der afrikanischen *Delavoiei* und der europäischen *vinula* charakterisiert werden. Ich erhielt 4 ♂♂ und 7 ♀♀ der reinen *albanica*, alle nach einmaliger Überwinterung. Es sind ungewöhnlich grosse Tiere, die in beiden Geschlechtern einen schmutzig bräunlichen Farbenton aufweisen. Die Flügel des Männchens sind kaum durchsichtiger als diejenigen des Weibchens und in der Farbe auch nicht heller. Im Vergleich mit *Delavoiei* sind dagegen die Zeichnungen schärfer hervortretend und von einer dunkleren schwarzgrauen Farbe.

Die Kreuzung *albanica* ♀ × *germanica* ♂ ergab nur 2 ♂♂ und 1 ♀, die alle der Mutterform ähnlicher waren.

Erminea ♀ × *vinula* ♂. — Das Material dieses Bastards erhielt ich von Herrn KURT JOHN, der die Kreuzung ausführte und mir die kreuzbefruchteten Eier zusandte.

Die Raupen sowie die Puppen der beiden Arten sind einander so ähnlich, dass sie bei einem oberflächlichen Vergleich nicht von einander zu trennen sind. Einer eingehenden Untersuchung habe ich sie nicht unterworfen. Die Eier sind dagegen von ganz verschiedenem Typus, und die Imagines unterscheidet man auch sofort. Der Bastard ist im Imagostadium intermediär. Er tritt viel häufiger im männlichen als im weiblichen Geschlecht auf. Vermutlich handelt es sich hier um einen ähnlichen Fall wie bei dem Bastard *Smerinthus populi* ♀ × *ocellata* ♂, der jedoch ausschliesslich im männlichen Geschlecht auftritt (FEDERLEY, 1933). Bei dem Bastard *erminea* ♀ × *vinula* ♂ sind einige Weibchen

bekannt; in meiner Zucht kamen jedoch keine Weibchen zur Entwicklung. Es ist anzunehmen, dass das X-Chromosom von *vinula* in seiner Wirkung so kräftig ist, das es zusammen mit dem Y-Chromosom von *erminea* in der Regel ein Männchen ergibt.

Erminea ♀ × (*erminea* ♀ × *vinula* ♂) ♂. — Die Kopula wurde nicht beobachtet. Es legten aber die *erminea* ♀♀, die mit den Bastard ♂♂ zusammen gewesen waren, zahlreiche Eier ab, von denen 65 Raupen ergaben. Die allermeisten dieser Raupen gingen an einer Darmkrankheit zu Grunde. Es wurden jedoch einige Puppen erhalten, von denen zwei für Fixierung der Testes geopfert wurden. Die zytologischen Untersuchungen sprechen dafür, dass hier mit grosser Wahrscheinlichkeit eine Rückkreuzung tatsächlich stattgefunden hat.

Vinula ♀ × (*erminea* ♀ × *vinula* ♂) ♂. — Diese Rückkreuzung ist mir zwei Mal gelungen. Die beiden Weibchen legten zahlreiche Eier ab. Aus dem einen Gelege von Hunderten von Eiern entwickelte sich kein einziges. Das andere, das auch sehr zahlreiche Eier enthielt, ergab eine einzige Raupe, die jedoch im vierten Stadium starb.

III. DIE ZYTOLOGISCHEN UNTERSUCHUNGEN.

Die *Dicranura*-Rassen bieten für zytologische Untersuchungen gewisse Vorteile. Die Zahl der Chromosomen ist im Vergleich mit derjenigen der meisten Lepidopteren verhältnismässig gering und die Grösse derselben recht erheblich. Dazu kommt dass die verschiedenen Stadien der Spermatogenese in der Regel sehr klar hervortreten und die Reifeteilungen von grosser Regelmässigkeit sind und demzufolge ausgezeichnet klare Bilder bieten.

Die Reifeteilungen der Spermatogenese finden erst im Puppenstadium statt. Die erwachsene Raupe enthält leider keine Reifeteilungen — wie dies dagegen bei anderen verwandten Bombyciden der Fall ist — was sehr zu bedauern ist, da die meisten meiner Raupen als erwachsen starben, und ich demzufolge kein zytologisches Material erhalten konnte. Die Reifeteilungen beginnen offenbar gleichzeitig mit der imaginalen Metamorphose. Es empfehlt sich deshalb die Puppen im Winter für einige Tage bis zwei Wochen ins Zimmer zu nehmen und erst dann den Testis zu fixieren. Die Fixierung erfolgte in der Regel in dem Gemisch von CARNOY (6 : 3 : 1). Meistens enthält ein solcher Testis alle Stadien der Spermatogenese. Allem nach zu urteilen findet die Spermatogenese bei den mehrmals überwinternden Puppen in mehreren Perioden statt, denn auch die zweijährigen Puppen wiesen

Reifeteilungen auf, enthielten aber eine grössere Anzahl reife Spermien, von denen vermutlich die allermeisten während des ersten Frühlings der Puppenzeit gebildet worden waren.

Die Reifeteilungen im Ei geschehen kurz nach dem Ablegen desselben. Eier, die 1—1 $^1/_2$ Stunden nach der Ablage fixiert worden sind, zeigen meistens die Metaphase der 2. Reifeteilung. Die Platten der ersten Reifeteilung erhält man am besten in reifen, noch nicht oder ganz frisch abgelegten Eiern. Da die Eischale sehr hart und dick ist, und die Mikropyle die Fixierungsflüssigkeit nur schwer durchzulassen scheint, muss die Eischale in der Fixierungsflüssigkeit perforiert werden. Die Äquatorialplatten liegen in der Regel tangential zur Oberfläche des Eies.

Vor dem Einbetten der Eier in Parovax muss die Eischale entfernt werden, da das Schneiden der Eier sonst unmöglich ist. Das Abpräparieren der Schale bietet bei den in CARNOYs Gemisch fixierten Eiern keine Schwierigkeiten, weil der Eidotter sich zusammenzieht und eine zähe Konsistenz erhält. Nach Behandlung der Eier mit verschiedenen anderen Fixierungsmitteln ist dies nicht der Fall, und die Entfernung der Schale wird unmöglich. Bei Fixierung nach der Methode von PETRUNKEWITSCH, von SEILER ganz besonders für Schmetterlingseier empfohlen, zieht sich der Eidotter nicht zusammen und haftet sehr oft an der Schale, die sich also nicht ablösen lässt ohne dass das Ei beschädigt wird. In Eiern, die in FLEMMINGs Gemisch fixiert worden sind, bildet der Dotter eine bröcklige Masse, die bei den Versuchen die Schale abzupräparieren vollständig zerfällt.

Die Mitosen der Blastodermzellen finden bei einer Temperatur von etwa 20° C ca. 20 Stunden nach der Eiablage statt. Sie liefern oft sehr klare Platten, in denen die Zahl der Chromosomen einigermassen exakt festgestellt werden kann.

1. DIE REINEN ARTEN UND VARIETÄTEN.

A. DICRANURA ERMINEA ESP.

Das Material dieser Art habe ich aus Deutschland erhalten und in Finnland ab ovo gezüchtet. Nur Testes von zwei Individuen wurden untersucht.

a. *Spermatogenese.* — Die Art ist für ein Studium der Spermatogenese äusserst günstig. Fast alle Stadien treten sehr schön, teilweise sogar schematisch klar hervor. Die Spermatogonienteilungen sind so deutlich, dass man die diploide Chromosomenzahl annähernd bestimmen kann.

In einer Platte gelang es sogar die Zahl als exakt *56* festzustellen. Das leptotäne Stadium ist von kurzer Dauer. So bald die anfangs körnerähnlichen Chromosomen der jungen Spermatozyten in die Länge wachsen, verlieren sie ihre Färbbarkeit und erscheinen erst deutlicher, wenn die Synizesis eintritt. Hier kann man indessen in einzelnen Kernen die Synapsis der Chromosomen verfolgen. Offenbar findet sie sehr schnell statt. Zuweilen entstehen typische Bukettstadien, aber meistens ragen die Enden von 1—4 Chromosomen aus dem lockeren Knäuel hervor und strecken sich bis zur entgegengesetzten Kernwand. Sehr oft kann man gerade an diesen langen freiliegenden Chromosomen die beiden Konjuganten unterscheiden und zuweilen kommt ein Strepsinemastadium zur Entwicklung. Die Zysten mit der exzentrischen Anhäufung der Chromosomen sind zahlreich und leicht erkennbar, denn sie liegen immer konzentrisch um die VERSONsche Zelle. Die Chromosomen verteilen sich sodann gleichmässig über den Kern, und es folgt das pachytäne Stadium, das ganz besonders klar ist. Ein diplotänes Stadium kann dagegen nur äusserst selten gefunden werden, denn die Achromatisierung erfolgt sehr schnell. Eine zweite Kontraktion (second contraction) habe ich in den zwei von mir untersuchten Testes nicht finden können. In der Diakinese sind die Chromosomen nicht so rein und klar wie dies z. B. bei den nahe verwandten *Pygaera*-Arten der Fall ist. Es haften allerhand gefärbte Brocken an den Chromosomen, wodurch ein eingehendes Studium der Tetraden — Kreuze und Ringe — sehr erschwert wird.

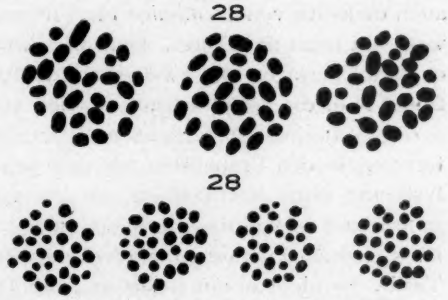


Fig. 1. *erminea*. Spermatozytenplatten I und II, alle mit 28 Chromosomen.

Die Reifeteilungen (Fig. 1) sind äusserst klar und regelmässig. Die grossen Chromosomen sind von verschiedenen Grössestufen, aber weder besonders grosse noch extrem kleine kommen vor; sie bilden eine Serie ohne scharfe Übergänge. In den Anaphasen wandern die Chromosomen ziemlich synchron zu den Polen. In einigen Anaphasen war es möglich an den grossen Chromosomen und zwar in beiden Enden eine interzonale Faser (Stemmkörper nach BELAR) zu entdecken. Die eigentlichen »Zugfasern» sind dagegen weniger scharf hervortretend. Zuweilen konnte Nicht-Trennen eines Chromosomenpaares festgestellt

werden, und in ganz seltenen Fällen bleibt ein Fragment eines Chromosoms in der Anaphase in der Spindelmitte zurück.

Die Chromosomenzahl ist ausnahmslos *28*. Eine Zählung der besten Platten in den beiden untersuchten Testes ergab folgendes Resultat: 132 Platten I und 44 Platten II, alle mit 28 Chromosomen.

Die *Oogenese* und die *Blastodermmitosen* habe ich nicht Gelegenheit gehabt zu untersuchen.

B. DICRANURA VINULA L. HAUPTFORM = GERMANICA.

Mein Material von dieser Form stammt teils aus Böhmen, Steinschönau, teils aus Schlesien, der Umgebung von Breslau. Ein Testis von jedem Fundort wurde untersucht.

a. *Spermatogenese.* — Wie die Spermatogenese von *erminea* ist auch diejenige von *germanica* für Untersuchungen äusserst dankbar, ja vielleicht noch dankbarer, denn die Chromosomen sind hier erstens an der Zahl geringer und zweitens in der Grösse erheblicher. Auch hier findet man die verschiedenen Stadien in schöner Klarheit.

Die Anaphasen der ersten Reifeteilung sind von besonderem Interesse. In den Präparaten von dem schlesischen Testis, bei deren Anfertigung keine Nachfärbung mit Plasmafarben zur Anwendung kam, kann man — wie dies meistens an gut fixierten Präparaten der Fall ist — keine »Zugfasern» entdecken. Die Präparate vom böhmischen Testis, der nicht so gut fixiert und mit Tiazinrot nachgefärbt ist, zeigen dagegen sehr deutliche Fibrillen und Strahlungsfiguren, wie dies bekanntlich in nicht gut fixierten Präparaten gewöhnlich ist. In gewissen Zellen treten die »Zugfasern» ziemlich klar hervor, und man kann in klaren Anaphasen I ausserdem ganz deutlich zwei an jedem Chromosom befestigte interzonale Fasern sehen. Wir kommen später auf die Bedeutung dieser Tatsache zurück.

Wie gesagt sind die Chromosomen bei *germanica* bedeutend grösser als bei *erminea* und ihre haploide Zahl beträgt ausnahmslos *21* (Fig. 13). In den beiden Testes habe ich diese Zahl in zusammengezählt 109 Platten der ersten und in 88 Platten der zweiten Reifeteilung exakt feststellen können. In der Regel kann man zwei kleine Chromosomen deutlich durch ihre geringe Grösse von den übrigen unterscheiden.

b. *Oogenese.* — Die Oogenese bietet nichts eigentümliches und weicht in keiner Hinsicht von derjenigen der folgenden Form ab. Ich habe sie deshalb nur ganz vorbeigehend untersucht um das importierte Material zu sparen. Die Chromosomenzahl ist auch hier 21.

c. *Parthenogenese.* — In zahlreichen Eiern von zwei Weibchen aus

Sachsen-Altenburg, die von finnländischen Männchen befruchtet worden waren, habe ich eine beginnende Parthenogenese und zwar eine haploide solche konstatieren können. Von denselben Weibchen gleichzeitig abgelegte Eier zeigten auch eine normale beginnende Entwicklung nach regelrechter Befruchtung.

Die Eier mit parthenogenetischer Entwicklung waren 3 $^1/_2$—4 Stun-

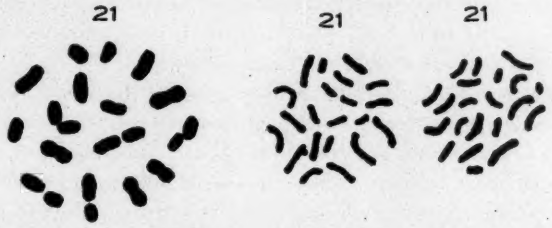


Fig. 2. *vinula fennica.* Oozytenplatte I. — *vinula germanica.* Zwei Platten aus einem parthenogenetischen Ei.

den nach dem Ablegen fixiert. In diesen findet man meistens, jedoch nicht immer, die drei Polkerne. Wenn sie vorhanden sind, können sie entweder jeder für sich oder alle zusammen, in einen Riesenkern vereinigt, in Teilung begriffen sein. Es ist also klar, dass in diesen Eiern die normalen Reifeteilungen stattgefunden haben, aber die Vereinigung des männlichen und weiblichen Pronukleus ist aus irgendeinem Grunde ausgeblieben, und einer von den beiden hat angefangen sich zu teilen. Denn in solchen Eiern findet man immer eine gewisse Anzahl Kerne, die sich teilen und in denen man die haploide Chromosomenzahl 21 exakt feststellen kann. Es kommen aber auch Eier vor, in denen man vergeblich nach den Polkernen sucht und man trotzdem eine Anzahl Mitosen mit 21 Chromosomen entdeckt. Die Form der Chromosomen in diesen haploiden Kernen ist eine andere als diejenige in den Meioseteilungen. Die Chromosomen sind nicht wie in der Meiose kurz und oval, sondern länglich, sogar länger als in den Blastodermmitosen. Man vergleiche die Chromosomen der Fig. 2 mit denjenigen der Fig. 3, die somatische Chromosomen im Blastoderm vorstellen, und denjenigen der Fig. 14, die Chromosomen der zweiten Reifeteilung abbilden. Die Form der Chromosomen der parthenogenetischen Kerne sind deutlich länger als diejenigen der Blastodermmitosen. In der Metaphase der partheno-

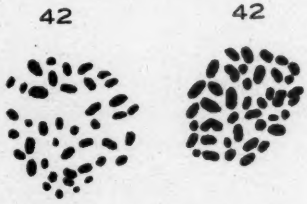


Fig. 3. *fennica.* Blastodermmitosenplatten.

genetischen Kerne sind die Zentrosomen oft sehr deutlich, was also auch beweist, dass es sich nicht um eine Reifeteilung handelt.

Es kann also keinem Zweifel unterliegen, dass eine parthenogenetische Entwicklung in den Eiern eingeleitet worden ist. Wie weit sich die Eier entwickelt hätten, ist dagegen unmöglich zu sagen. Die höchste Anzahl Kerne, in denen ich mit vollständiger Sicherheit die haploide Chromosomenzahl habe feststellen können, ist 10—12. Daneben kamen aber eine Anzahl Kerne vor, die sich im Ruhestadium befanden und mit aller Wahrscheinlichkeit auch haploid waren.

Bei *Pygaera*-Bastarden habe ich haploide Blastodermzellen nachweisen können (FEDERLEY, 1931), was also beweist, dass die parthenogenetische Entwicklung bis zur Bildung des Blastoderms fortschreiten kann. Es scheint übrigens eine gewisse Tendenz zur Parthenogenese bei vielen Schmetterlingen vorzuliegen, wie u. a. die Züchter von *Bombyx mori* öfter festgestellt haben und SEILER seinerzeit hervorgehoben hat.

C. DICRANURA VINULA L. VAR. FENNICA SCHULTZ.

Von dieser in Finnland allgemein vorkommenden Form habe ich mehrere Individuen untersucht. Sie stammen aus verschiedenen Teilen des Landes und sind untereinander in zytologischer Hinsicht ausserordentlich ähnlich. Sie unterscheiden sich auch nicht von der mitteleuropäischen Form *germanica*.

a. *Spermatogenese.* — Alle vier untersuchten Testes stammen aus 2-jährigen Puppen und enthalten wohl aus diesem Grunde verhältnismässig wenige Reifeteilungen. Ich gebe hier eine Zusammenstellung der von mir gefundenen Chromosomenzahlen.

		Chromosomenzahlen			
		20	*21*	*22*	
Tammerfors, Birkkala 1 Spermatozyten	I		3		
»	II		12		
» » 2 »	I	2	19		
»	II		11		
Südfinnland »	I		4		
»	II	1	13		
» »	I	2	78	1	
»	II		59		

Wie aus den obigen Zahlen hervorgeht, ist 21 die für *fennica* charakteristische Zahl. Die Zahlen 20 und 22 sind ganz seltene Ausnahmen.

b. *Oogenese.* — Beide Reifeteilungen sind meistens deutlich, und in beiden kann man 21 als die haploide Zahl feststellen (Fig. 2).

c. *Blastoderm.* — Die Mitosen sind verhältnismässig klar, und in vielen Platten kann man ganz exakt 42 Chromosomen zählen (Fig. 3).

D. DICRANURA VINULA L. ALBANICA.

Von dieser Form lagen nur ein Testis und Eier von zwei Weibchen vor.

a. *Spermatogenese.* — Der Testis ist etwas älter als diejenigen von *fennica* und *germanica* und enthält deshalb keine prämeiotischen Stadien. Auch die Spermatogonienteilungen sind spärlich und eine exakte

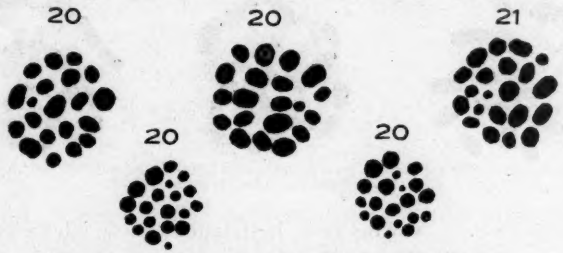


Fig. 4. *albanica.* Spermatozytenplatten I und II.

Zählung der Chromosomenzahl ausgeschlossen. Dagegen kommen noch Reifeteilungen ziemlich reichlich vor. Eine sorgfältige Durchmusterung der Platten in bezug auf die Chromosomenzahl ergab folgendes Resultat:

		Chromosomenzahlen			
		19	*20*	*21*	*22*
Spermatozyten	I		81	13	6
»	II	2	42	2	3

Die überwiegende Mehrzahl der Platten oder 123 von 149 enthält also 20 Chromosomen, weshalb wir die Behauptung wagen können, dass *20* die haploide Chromosomenzahl des Männchens dieser Form ist.

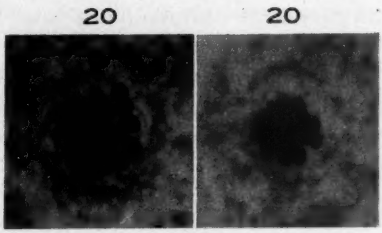


Fig. 4 *a.* *albanica.* Spermatozytenplatte I und II.

Wie die Fig. 4 zeigt, sind die Chromosomen etwas grösser als diejenigen von *germanica* und *fennica* und ihre Form ist eine rundere. Sie liegen auch mehr zusammengedrängt in der Platte als bei den letztgenannten Formen. Ich war zunächst geneigt diese Merk-

male als für *albanica* charakteristisch zu betrachten, da alle Zysten ähnliche Chromosomentypen enthalten. Jetzt möchte ich die abweichende Form als etwas rein phänotypisches bezeichnen. Ich habe nämlich in einem Testis des Bastards *fennica* × *germanica* zwei Zysten entdeckt, die ganz nebeneinander liegen und von denen die eine die für *fennica* und *germanica* charakteristischen kleineren, etwas länglicheren und weiter von einander liegenden Chromosomen zeigt, die andere dagegen die für den eben beschriebenen *albanica*-Testis typischen Chromosomen besitzt.

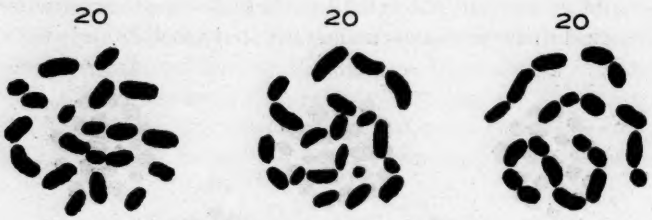


Fig. 5. *albanica*. Oozytenplatten I.

b. *Oogenese*. — Nur die erste Reifeteilung hatte ich Gelegenheit zu untersuchen. In 15 Eiern konnte die Chromosomenzahl festgestellt werden. In 11 von diesen beträgt sie exakt *20* (Fig. 5), in 3 war es nicht möglich die Zahl ganz einwandfrei zu konstatieren, denn die Platten waren durch das Messer in zwei Schnitte zerlegt. Es scheint mir jedoch fast sicher, dass auch diese Platten 20 Chromosomen enthalten hatten. In einer Platte konnten schliesslich nur 19 Chromosomen gezählt werden.

Es ist also klar, dass beide Geschlechter von *albanica* 20 Chromosomen haben, obgleich bei der Spermatogenese eine Fragmentation vorkommen kann, die die Zahl auf 21 erhöht, ja sogar in seltenen Fällen eine Erhöhung bis 22 verursachen kann. Wie die abgebildete Spermatozyte mit 21 Chromosomen zeigt (Fig. 4), enthalten diese Platten ein überzähliges kleines Chromosom.

Ob die wenigen Platten mit 19 Chromosomen das Resultat einer Fusion von zwei Chromosomen sind, wage ich nicht zu entscheiden; vieles deutet jedoch daraufhin, dass dies der Fall ist.

E. DICRANURA VINULA L. VAR. DELAVOIEI GASCHET.

Das Material dieser Form, eine Anzahl Puppen, wurde mir von Herrn KURT JOHN geliefert und stammte angeblich aus Marokko. Wegen des unregelmässigen Ausschlüpfens der Falter wagte ich es nicht das

spärliche mir zur Verfügung stehende Material zu vermindern und fixierte deshalb keine Testes. Ich hegte die Hoffnung *Delavoiei*-Zuchten zu erhalten, und meine Absicht war die Spermatogenese an Puppen dieser Zuchten zu studieren. Leider gingen die erwachsenen Raupen alle ein, wodurch meine Pläne vereitelt wurden. Es ist mir deshalb nur möglich Angaben über die Chromosomen der Oogenese und des Blastoderms zu geben.

a. *Oogenese.* — In der ersten Reifeteilung kann man exakt *31* Chromosomen zählen, die im Vergleich mit denjenigen von *vinula* bedeutend

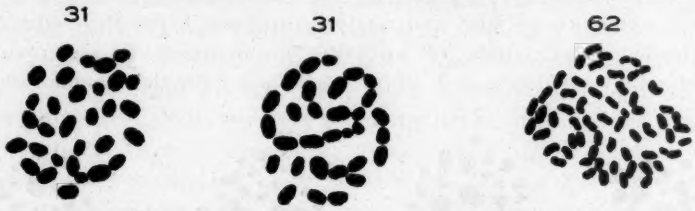


Fig. 6. *Delavoiei.* Oozytenplatten I und Blastodermmitosenplatte.

kleiner sind. Untereinander verglichen sind sie recht verschieden gross. Die Fig. 6 zeigt uns zwei solche Platten.

Von der zweiten Reifeteilung ist es mir nicht gelungen klare Bilder zu erhalten.

b. *Blastoderm.* — Die Blastodermzellen enthalten *62* kleine, längliche Chromosomen, die keine auffallenden Grössenunterschiede aufweisen (Fig. 6).

Man ist also berechtigt anzunehmen, dass das Männchen von *Delavoiei* die haploide Zahl 31 besitzt.

2. DIE INTERSPEZIFISCHEN BASTARDE.

A. D. ERMINEA ♀ × VINULA ♂.

Von diesem Bastard habe ich, wie gesagt, lauter Männchen erhalten. Die Spermatogenese habe ich in ihren Hauptzügen in einer kurzen Mitteilung (FEDERLEY, 1915 b) geschildert, wobei ich mich besonders bei der Konjugation der artfremden Chromosomen und ihrer Neigung mit einander zu verschmelzen aufhielt. Ich werde auf diese Fragen später zurückkommen.

a. *Spermatogenese.* — Die Testes der erwachsenen Raupen enthalten, ganz wie diejenigen der Elternarten, in dem entsprechenden Stadium sehr schöne Synapsis-, Synizesis- und Pachytänstadien. Besonders die letzterwähnten sind sehr klar und schön. Es findet also eine

Chromosomenzahlen der Spermato-

	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Hoden A	1		3	3	4	6	9	17	10	21	10	15	15	9	4	1	2	1
» B									3	2		2	1		3	2		1

vollständig regelrechte Konjugation der artfremden Chromosomen statt, die jedoch schon längst vor der ersten Reifeteilung für eine grössere oder geringere Anzahl aufgehoben ist und auf solche Weise ohne Wirkung wird.

Aus drei Puppen standen mir die Hoden zur Verfügung. Von diesen war einer zu jung und zeigte demzufolge keine Reifeteilungen. Die beiden anderen habe ich sorgfältig durchmustert; da sie untereinander sehr verschieden sind, werde ich sie jeder für sich behandeln.

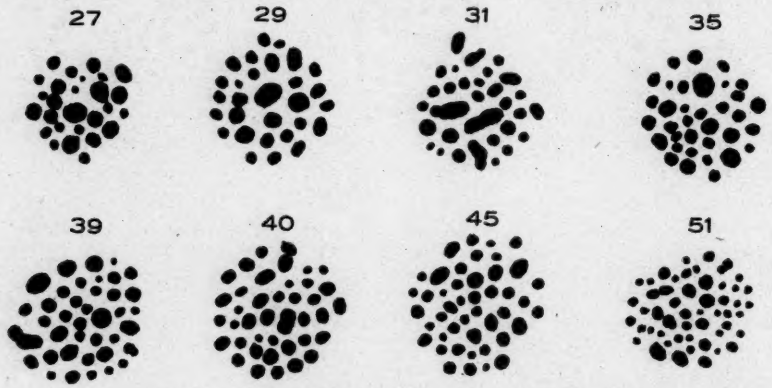


Fig. 7. *erminea* × *vinula*. Spermatozytenplatten I.

Hoden A. — Dieser ist der jüngere Hoden. Hier kommen noch Synapsis- und Pachytänstadien in ziemlich grosser Anzahl vor. Auch vereinzelte Kerne im zygotänen Stadium konnten entdeckt werden, obgleich sie selten sind und keine besonders klare Bilder bieten. Die prämeiotischen Stadien machen in jeder Hinsicht einen vollständig normalen Eindruck. Erst in der ersten Reifeteilung zeigen sich die durch die Kreuzung hervorgerufenen Anomalien.

In der ersten Reifeteilung findet man eine lange Serie von Chromosomenzahlen, die von 27 bis 51 reicht. Wie bei den Lepidopterenbastarden überhaupt nimmt die Grösse der Chromosomen mit ihrer zunehmenden Anzahl ab. Davon überzeugt uns die Fig. 7. *Je zahlreicher die konjugierten Chromosomen sind, desto geringer ist die totale Anzahl der Chromosomen und desto erheblicher ihre Grösse.* Aber die

zyten I von erminea × vinula.

45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	N	M
1						1														133	36,98
	1			5	1	1	1				2		1	1					1	28	45,71

Erklärung, dass die Anzahl der konjugierten Chromosomen die totale Zahl der Chromosomen in der Platte bestimmt, ist nicht allein befriedigend. Denn wenn dies der Fall wäre, so müsste die Zahlenreihe ihr Minimum bei 28 haben, hier hätten also alle 21 *vinula*-Chromosomen mit der entsprechenden Anzahl *erminea*-Chromosomen konjugiert, und das Maximum würde 49 sein, denn diese Zahl ist die Summe der *vinula*- und *erminea*-Chromosomen. Nun kommen aber in der Serie eine Platte mit 27 und eine mit 51 Chromosomen vor, welche Zahlen also ausserhalb der Werte 28—49 liegen, und wie wir sehen werden, findet man in dem Hoden B noch weit höhere Zahlen. Es müssen also noch andere Faktoren dabei sein, die die Zahl der Chromosomen bestimmen. Die Entstehung der Zahlen unter 28 könnte man sich so vorstellen, dass zwei *erminea*-Chromosomen mit einem *vinula*-Chromosom konjugieren, eine Vorstellung, die berechtigt erscheint, da wir annehmen müssen, dass der Genbestand der beiden einander nahestehenden Arten keine grossen Unterschiede zeigt. Schwieriger ist dagegen die Bildung von Platten mit mehr als 49 Chromosomen zu erklären. Hier bleibt wohl kaum etwas anderes übrig als nach der viel verketzerten Fragmentierung und zwar von den grossen *vinula*-Chromosomen zu greifen, um so mehr da wir sehen werden, dass der Hoden B ganz besonders hierzu auffordert.

Hoden B. — Hier liegen die Verhältnisse ganz anders als in dem soeben beschriebenen Hoden A. Wie die Tabelle zeigt, ist die Zahlenreihe weit nach rechts verschoben und schwankt zwischen 35 und 64. Es scheint, als ob hätte die Konjugation hier seltener stattgefunden als in dem Hoden A, aber mit dieser Erklärung kommen wir nicht aus. Hier hat ausserdem eine Fragmentierung dazu beigetragen die Chromosomenzahlen zu erhöhen. Man könnte sich auch das Vorkommen von Tetraploidie vorstellen, worauf tetraploide Spermatogonien hindeuten. Man findet auch einzelne Spermatozyten mit vier Zentrosomen und einer grossen Anzahl kleiner Chromosomen, die nicht exakt gezählt werden können. Aber diese Erscheinungen sind Ausnahmen, und die überaus häufigen Platten mit zahlreichen kleinen Chromosomen, deren Zahl sicher 50 übersteigt, die jedoch hier in der Tabelle nicht eingeführt sind, sprechen für eine Fragmentierung.

Die zweite Reifeteilung ist weit schwieriger zu studieren und nur selten kann man hier exakte Zählungen anstellen. Im Hoden A habe ich die Zahl in 9 Platten festgestellt. Sie schwankte zwischen 33 und 40. In der Fig. 8 sind fünf dieser Platten abgebildet. Sehr oft sind die Schwesterplatten miteinander verbunden, so dass es nicht möglich ist zu entscheiden, zu welcher Platte die im Grenzgebiete liegenden Chromosomen zu zählen sind. In der Fig. 8 zeigt die Platte mit 37 Chromosomen eine solche Verbindung mit der Schwesterplatte, die im Verhältnis zu ihr rechtwinklig steht. In der Profilansicht dieser Spindel kann man sich auch von der wenig regelmässigen Orientierung der Chromosomen in der Äquatorialebene überzeugen.

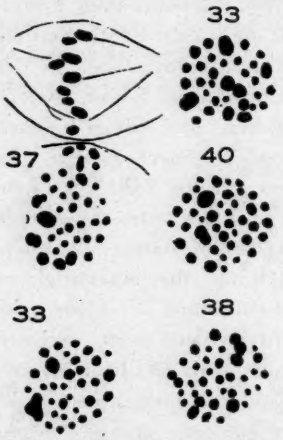


Fig. 8. *erminea* × *vinula*. Spermatozytenplatten II.

In der ersten Mitteilung (1915 b) über die Spermatogenese dieses Bastards habe ich die Vermutung ausgesprochen, dass die zusammengebrachten artfremden Chromosomen aufeinander schädlich einwirken und dass dieser schädliche Einfluss eine Verschmelzung oder Agglutination der Chromosomen verursacht, die offenbar von pathologischer Art ist und die Beurteilung der Konjugationsverhältnisse in hohem Grade erschwert. Ich habe in der genannten Mitteilung Metaphasen der ersten Reifeteilung sowohl als Platten als in Profilansichten abgebildet, in denen so riesengrosse Chromosomen vorkommen, dass es kaum möglich ist dieselben als normale konjugierte Chromosomen — seien sie auch plurivalent — aufzufassen. Diese Riesenchromosomen haben eine unregelmässige Form und liegen oft schräg in der Spindel, weshalb sie sofort auffallen. Auch unter den hier in der Fig. 7 abgebildeten Platten kommen Chromosomen vor, die kaum als normal bezeichnet werden können. In den Platten mit 31 und 39 Chromosomen sind diese unregelmässigen Chromosomen besonders in die Augen fallend. Wenn auch gern zugegeben werden kann, dass einige dieser langen Chromosomen durch Fusion zweier Chromosomen entstanden sein können, wie dies regelmässig und besonders klar bei dem intraspezifischen Mischling *fennica* × *germanica* zum Ausdruck kommt, so möchte ich trotzdem an meiner früheren Ansicht festhalten, dass die Mehrzahl dieser unregelmässigen Gebilde nicht mehr als normale Chromosomen aufzufassen

sind, sondern als durch die Kreuzung hervorgerufene pathologische Produkte. In der Anaphase I kann man nämlich diesen pathologischen Prozess weiter verfolgen. Er führt hier zu einer weitgehenden Agglutination der Chromosomen, die der Teilung den Charakter von einer Amitose verleiht. Ich betrachte also die vereinzelten agglutinierten Chromosomen der Metaphase als das Anfangsstadium dieses pathologischen Prozesses, der bei Artbastarden keine seltene Erscheinung ist. Der Prozess kann auch erst in der zweiten Reifeteilung einsetzen, wie die Platten II der Fig. 8 beweisen, von denen diejenige unten links einen besonders charakteristischen Klumpen zeigt.

Die Spermatogenese dieses Bastards zeichnet sich sonst durch allerhand Störungen aus, die für Speziesbastarde charakteristisch sind. Ausser den eben erwähnten pseudoamitotischen Reifeteilungen beobachtet man, dass die erste Reifeteilung nicht zum Abschluss kommt, sondern eine Art von Restitutionskern gebildet wird, oder dass die Teilung unvollständig erfolgt und die Interkinesekerne mehr oder weniger innig vereinigt werden, wie z. B. die Fig. 8 uns zeigt. Im ersten Fall können diploide Spermatozyten II entstehen, aber weit öfter resultiert die unvollständige Teilung in Bildung eines Tetrasters und einer ganz unregelmässigen Teilung. Sehr oft gelangen einige Chromosomen als Folge der unregelmässigen ersten Reifeteilung garnicht in den Bereich eines der beiden Schwesterspindel II und werden demzufolge eliminiert. Dies scheint nicht selten gerade mit den grossen Chromosomen zu geschehen, was als ein Zeichen ihrer pathologischen Natur gedeutet werden muss. Schliesslich erfolgen die Teilungen der Chromosomen nicht immer synchron, sondern einige hinken nach und werden deshalb leicht eliminiert.

B. D. ERMINEA ♀ × (ERMINEA ♀ × VINULA ♂) ♂.

Von diesem Rückkreuzungsbastard wurden die Hoden zahlreicher erwachsener kranken Raupen fixiert. Sie boten wenig von Interesse und sind mit denen des primären Bastards und der reinen Arten ganz übereinstimmend. Schöne pachytäne Stadien sind rings um die VERSONsche Zelle gruppiert. Leider sind die Spermatogonienteilungen äusserst selten und dazu infolge der nicht gelungenen Fixierung wertlos.

a. *Spermatogenese.* — Die Testes aus zwei Puppen befanden sich dagegen bei der Fixierung in einem sehr günstigen Stadium. Die Präparate enthalten eine grosse Anzahl Reifeteilungen, von denen auch die zweite so regelmässig verläuft, dass eine verhältnismässig sichere Bestimmung der Chromosomenzahl möglich ist. Ich gebe die gefundenen Chromosomenzahlen hier tabellarisch wieder.

Chromosomenzahlen der Reifeteilung I von erminea × (erminea × vinula).

	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	N	M
Anzahl der				1	1	2	9	4	6	25	14	18	13	9	5	1	108	41,88
Spermatozyten	1		3	5	4	10	10	16	13	13	4	11	2	4	1		97	39,54

Chromosomenzahlen der Reifeteilung II von erminea × (erminea × vinula).

Anzahl der					1	1	6	7	6	4	4	4	4	3	2		38	41,47
Spermatozyten	1				1		2	1									5	

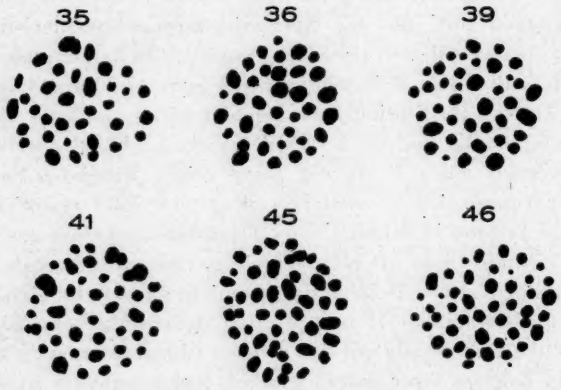


Fig. 9. *erminea* × (*erminea* × *vinula*). Spermatozytenplatten I.

Die Fig. 9 gibt einige Platten der ersten Reifeteilung wieder. Ein Vergleich dieser Platten mit denjenigen des primären Bastards (Fig. 7) zeigt erstens, dass die Grösseverhältnisse der Chromosomen andere sind. Die grossen Chromosomen des primären Bastards fehlen hier oder sind äusserst selten. Sie sind bei dem primären Bastard durch die Konjugation der grossen *vinula*-Chromosomen mit den kleineren *erminea*-Chromosomen entstanden, vielleicht sogar in der Weise, dass zwei *erminea*-Chromosomen mit einem *vinula*-Chromosom konjugiert haben. Bei dem Rückkreuzungsbastard kommen wohl in der Regel lauter bivalente *erminea*-Chromosomen und univalente *vinula*-Chromosomen vor. Ausserdem scheinen beim Rückkreuzungsbastard nur ganz selten agglutinierte Riesenchromosomen zu entstehen.

Zweitens zeigen die Zahlenserien und die Bilder der Platten des Rückkreuzungsbastards, dass die Frequenz der Chromosomenzahlen

bei diesem vom Vaterbastard abweicht. Der normal entwickelte Hoden A vom primären Bastard *erminea* × *vinula* zeigt die höchste Frequenzzahl bei 36 Chromosomen, sekundäre Gipfel bei 34 und 38—39 und ein $M = 36{,}98$. Der Hoden B ist durch seine zahlreicheren Anomalien und geringere Anzahl brauchbarer Reifeteilungen weniger gut für einen Vergleich geeignet. Beim Rückkreuzungsbastard fällt das Maximum bei dem einen Hoden bei 41 Chromosomen mit einem sekundären Gipfel bei 43 und $M = 41{,}88$, im anderen Hoden bei 39 mit einem sekundären Maximum auch bei 43 und $M = 39{,}54$. Ganz besonders muss hervorgehoben werden, dass der erste Hoden auch in der zweiten Reifeteilung ein $M = 41{,}47$ aufweist, was alles darauf hindeutet, dass diese Zahl in diesem Hoden kein Zufall ist.

In den Profilansichten der ersten Reifeteilung kann man öfter das für die Rückkreuzungsbastarde charakteristische Bild finden, indem bivalente semmelähnliche und univalente ovale Chromosomen nebeneinander in der Spindel stehen. Diese so typischen Bilder haben mich davon überzeugt, dass wir es hier tatsächlich mit einem Rückkreuzungsbastard zu tun haben.

Im übrigen kommen auch in den Hoden des sekundären Bastards ähnliche Anomalien wie bei dem primären vor. So sind tetraploide Spermatozyten II keine Seltenheit; auch vereinzelte tetraploide Spermatogonien sind verzeichnet worden. In den tetraploiden Zellen sind multipolare Mitosen eine allgemeine Erscheinung. Anachronismus kommt sowohl in der Entwicklung der einzelnen Zellen in einer Zyste vor als in der Teilung der Chromosomen einer Spindel. Spindeln, in denen die Chromosomen drei Gruppen bilden, eine in der Mitte, die beiden anderen in der Nähe der Polen, sind recht häufig.

C. D. VINULA V. DELAVOIEI ♀ × VINULA V. FENNICA ♂.

Dieser Mischling bietet die grösste Überraschung in bezug auf die Chromosomenverhältnisse. Nach den Erfahrungen an früher untersuchten Lepidopterenbastarden hätte man bei vollständiger Affinität zwischen den im Mischling zusammengebrachten Chromosomen die Zahl 31 erwartet, welche Zahl der grösseren der beiden Elternarten entspricht. Bei partieller oder total fehlender Affinität zwischen den *Delavoiei*- und den *vinula*-Chromosomen hätte die Zahl zwischen 32 und 52 schwanken müssen, weil die Summe der Chromosomenzahlen 52 beträgt. Keine von diesen Erwartungen trifft ein. Anstatt dessen finden wir die für *vinula* charakteristische Zahl 21 als die absolut häufig-

ste sowohl in der Spermato- als der Oogenese, eine Tatsache, auf deren theoretische Bedeutung wir in dem allgemeinen Teil zurückkommen.

a. *Spermatogenese.* — Aus erwachsenen Raupen herauspräparierte Testes zeigen, wie bei den reinen *Dicranura*-Arten und -Mischlingen überhaupt, eine beginnende Spermatogenese mit prämeiotischen Stadien, indem ringsum die VERSONsche Zelle Zysten mit Spermatozyten in den Synapsis- und Pachynemastadien stehen. Die Bilder sind sehr schön und klar, und von Abweichungen von dem normalen Verlauf der Spermatogenese ist nichts zu sehen.

In den Spermatogonien kommen häufig klare Mitosen vor, die zuweilen so klar sind, dass man in guten Platten die Zahl der Chromosomen exakt als 52 feststellen kann (Fig. 10). Da der Mischling von *Delavoiei* 31 und von *vinula* 21 Chromosomen erhalten hat, so war diese Zahl hier zu erwarten.

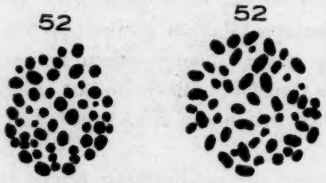


Fig. 10. *Delavoiei* × *fennica.* Spermatogonienplatten.

Nur zwei Testes mit Reifeteilungen standen mir zur Verfügung. Diese finden nämlich, wie gesagt, erst im Puppenstadium bei der beginnenden Metamorphose statt, und in der Hoffnung eine F_2-Generation erhalten zu können wollte ich nicht mehr als zwei Puppen für zytologische Zwecke opfern. Beide Puppen enthielten Reifeteilungen, leider jedoch in nicht sehr grosser Menge.

Das pachytäne Stadium ist sehr schön und klar wie bei den reinen Formen. Es ist mir nicht gelungen in diesem Stadium der Spermatogenese irgendwelche Veränderungen zu entdecken, die auf Störungen infolge der Bastardnatur der Testes hätten zurückgeführt werden können. Auch die Diakinese macht einen durchaus normalen Eindruck und zeigt die bei den reinen Formen vorkommenden Kreuze und Semmeln. Zwar habe ich in einigen Kernen eine verschiedene Länge der Konjuganten feststellen können. Besonders in solchen Zellen, die einen Übergang zwischen dem eupyrenen und dem apyrenen Typus bilden und in denen eine Konjugation, aber eine wenig feste solche, noch stattfindet, tritt diese Ungleichheit in der Länge der konjugierenden Chromosomen deutlich zu Tage. Da solche Übergangsstadien jedoch sehr selten sind, kommen derartige Bilder nur ausnahmsweise zu Gesicht. In den meisten eupyrenen Diakinesen ist nichts von einer Disharmonie der Gemini zu entdecken.

Die Reifeteilungen sind dagegen sehr auffallend, indem die aller-

meisten, wie gesagt, 21 grosse Chromosomen wie bei *vinula* aufweisen. Ist die Zahl eine grössere, so findet man in der Platte meistens ungefähr ebenso viele kleine Chromosomen, wie die Anzahl der Chromosomen 21 übersteigt. Man vergleiche die Abbildungen (Fig. 11). Die kleinen

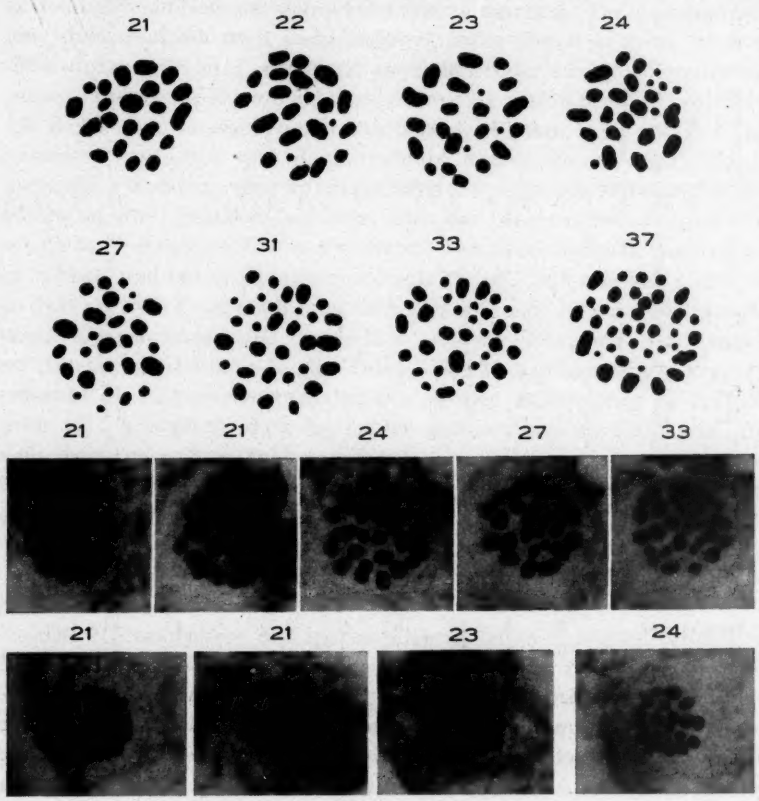


Fig. 11. *Delavoiei* × *fennica*. Spermatozytenplatten I und II.

Chromosomen sind vermutlich *Delavoiei*-Chromosomen, die mit den *vinula*-Chromosomen nicht konjugiert haben.

In der Tabelle habe ich die Chromosomenzahlen der einzelnen Spermatozyten der beiden Testes nach den Zysten geordnet, um zu zeigen, dass die Verhältnisse in den verschiedenen Zysten nicht gleich sind. In mehreren Abhandlungen (FEDERLEY, 1931, 1933) habe ich darauf hingewiesen, dass die Art und Weise der Konjugation nicht nur

Chromosomenzahlen der Spermatozyten I von Delavoiei × vinula.

Anzahl Spermatozyten in den Zysten A—L	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	N	
B		1		1	1														3	
C		1		1		1													3	
D		6	2																8	
E		3	1	1															5	
F		7	1	1															9	
G					2		1	3	2	1	1	1	1	2	1		1		16	
H		4	2																6	
I	1	2																	3	
K			2																2	
L		1																	1	
	1	25	8	4	3	1	1	3	2	1	1	1	1	2	1		1	56		M = 23,78
A		7	3	2															12	
B		2																	2	
C		7		1	1														9	
D		5	1																6	
E		7	3	1															11	
F		6	1		1														8	
G		5																	5	
H		10	2	2															14	
I		3		1															4	
K		2																	2	
L		4	2																6	
		58	12	7	2														79	M = 21,41

Chromosomenzahlen der Spermatozyten II von Delavoiei × vinula.

	21	*22*	*23*	*24*	*25*	*26*	*27*	*28*	N
Anzahl der	6	9	10	6	6	3		4	44
Spermatozyten	10	9	10	4	2				35

von den Genen der Chromosomen, sondern auch von den Umweltbedingungen bestimmt wird. Dies scheint mir besonders klar aus der Tabelle hervorzugehen. Die Zyste *G* mit ihren 16 Platten weist alle die hohen Zahlen auf. Diese variieren zwischen 24 und 37. Die übrigen 9 Zysten mit ihren 40 zählbaren Platten haben nur eine mit je 24, 25 und 20 Chromosomen, die übrigen haben die Zahlen 21—23, und nicht weniger als 25 zeigen die Zahl 21. Es scheint mir ganz offenbar, dass die Zyste *G* einer Konjugation weniger günstige Bedingungen geboten hat als die übrigen Zysten es getan haben.

Wie immer in einem Bastardtestis weist die zweite Reifeteilung auch hier grössere Unregelmässigkeiten auf als die erste. Man findet hier eliminierte Chromosomen, Zellen mit vier Zentrosomen und demzufolge Tetrasterbildung, diploide Chromosomenzahlen und allerhand

pathologische Verhältnisse. Alles zeigt klar, dass wir es mit einem Speziesbastard zu tun haben.

Das Zählen der Chromosomen in der zweiten Reifeteilung bietet weit grössere Schwierigkeiten, denn die kleinen *Delavoiei*-Chromosomen zeigen hier eine Neigung sich wieder frei zu machen. Da die konjugierten Chromosomen noch dicht nebeneinander liegen können, wird es demzufolge schwierig, oft ganz unmöglich oder einfach Geschmacksache, zu entscheiden, ob nur ein oder zwei Chromosomen vorliegen. Als Regel kann angegeben werden, dass die Platten II 21 grosse Chromosomen enthalten und dazu mehrere kleine, deren Anzahl exakt anzugeben meistens eine Unmöglichkeit ist. Zu der schwierigen Bestimmung ihrer exakten Zahl trägt noch der Umstand bei, dass sie sehr oft nicht in derselben Ebene wie die grossen Chromosomen liegen, sondern ober- und unterhalb dieser und, wie gesagt, dicht an diese gedrückt, so dass sie schwer zu entdecken sind. Dass es sich hier um die grossen *vinula*-Chromosomen und die kleinen *Delavoiei*-Chromosomen handelt scheint mir ganz klar. In der Tabelle S. 228 habe ich einen Versuch gemacht die Chromosomenzahlen der zweiten Reifeteilung zu ordnen, muss jedoch zugeben, dass die Zahlen gewissermassen relativ sind, indem man oft andere Zahlen angeben könnte, je nachdem man zwei dicht aneinander liegende Chromosomen, ein grosses und ein kleines, als ein oder zwei zählt. In sehr vielen und klaren Platten kann indessen die Zahl 21 fortwährend ganz exakt und eindeutig festgestellt werden, und dies scheint mir das wichtigste und am meisten Aufsehen erregende zu sein.

Die Metamorphose der Spermatiden in reifen Spermien scheint in den verschiedenen Zysten verschieden zu erfolgen. Neben zahlreichen Bündeln von äusserlich vollständig normalen Spermien — allem nach zu urteilen solchen, die 21 Chromosomen enthalten — entdeckt man jedoch auch zahlreiche degenerierende Zysten, die zerfallende Spermatiden enthalten. Ich sehe hier selbstverständlich von den apyrenen Spermien ab, die ja immer auch bei den reinen Arten vorkommen und auch nicht bei den Bastarden fehlen.

Chromosomenzahlen der Oozyten I von Delavoiei × vinula.

	19	*20*	*21*	*22*	*23*	*24*	*25*	*26*	N	M
Anzahl der Oozyten	1	1	25	2	4	1		2	36	21,5

b. *Oogenese.* — Die Eier von drei Weibchen wurden fixiert. Da leider keine Männchen zur Verfügung standen, konnte keine Befruchtung der Eier erzielt werden und demzufolge nur die Metaphase der

ersten Reifeteilung erhalten und untersucht werden. Wie schon hervorgehoben wurde, bietet die Fixierung der Eier Schwierigkeiten, und diese zeigten sich gerade bei diesem wertvollen Material besonders ärgerlich, indem die Neigung der Chromosomen sich zu Ketten zu vereinigen sehr stark hervortrat. Nur wenige ganz einwandfreie Platten wurden er-

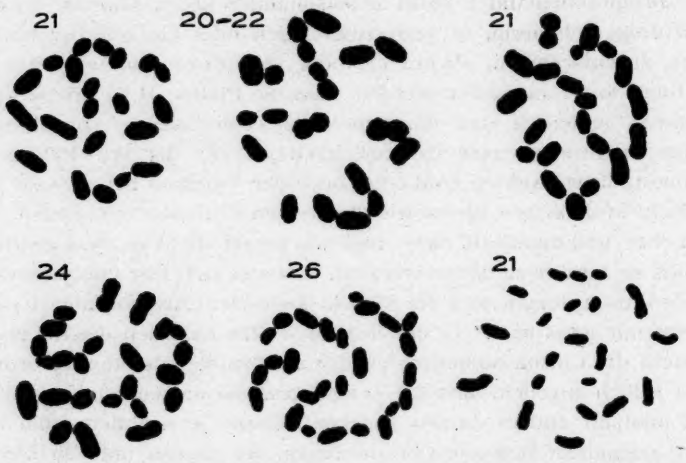


Fig. 12. *Delavoiei* × *vinula*. Oozytenplatten I. In der Platte oben in der Mitte kann die Chromosomenzahl als 20 oder 22 angegeben werden, je nachdem man die zwei semmelförmigen Chromosomen jedes als ein bivalentes oder als zwei selbständige verklebte Chromosomen auffasst.

halten, von denen die klarsten in der Fig. 12 abgebildet sind. Wie die Tabelle Seite 229 zeigt, enthalten die meisten Platten 21 Chromosomen und die höheren Zahlen sind überhaupt selten. Jedoch muss zugegeben werden, dass die Zahlen nicht ganz exakt sind, da man infolge der starken Verklebung der Chromosomen nicht immer die einzelnen Chromosomen ganz sicher von einander unterscheiden kann.

3. DIE INTRASPEZIFISCHEN BASTARDE.

A. D. VINULA V. ARCTICA ♀ × VINULA GERMANICA ♂.

Nur ein einziges F_2-Weibchen dieser Kreuzung wurde fixiert.

a. *Oogenese.* — In vier ganzen Platten der ersten Reifeteilung konnte die Chromosomenzahl sicher als *21* festgestellt werden, und in vier weiteren, die auf zwei Schnitte verteilt waren, konnten gleichfalls *21* Chromosomen gezählt werden.

Man kann wohl annehmen, dass *arctica* wie *vinula* 21 Chromosomen hat, und dass diese Zahl bei dem Bastard beibehalten wird.

B. D. VINULA V. FENNICA ♀ × VINULA GERMANICA ♂.

Die Kreuzung *fennica* ♀ × *germanica* ♂ habe ich zweimal ausgeführt, das erste Mal 1914 zwischen einem Weibchen aus dem Kirchspiel Pojo in Finnland und einem Männchen aus Steinschönau in Böhmen, das zweite Mal 1933 mit einem Weibchen aus Pernå in Finnland und einem Männchen aus Breslau, Schlesien. Die zytologischen Untersuchungen einer Anzahl Mischlinge aus diesen Kreuzungen ergaben für verschiedene Individuen verschiedene Resultate und sind in mancher Beziehung überraschend und schwer zu deuten.

a. *Spermatogenese.* — Die Chromosomenzahl der Spermatogonien habe ich in keinem Fall absolut exakt feststellen können. Die prämeiotischen Stadien sind in den meisten Präparaten ebenso schön wie bei den Elternformen. Erst während der Meioseteilungen können in einigen Testes Anomalien verzeichnet werden, die darauf hindeuten, dass in dem vorliegenden Fall keine regelrechte Kopula zwischen Individuen derselben Art und Rasse stattgefunden hat, sondern dass eine Verbindung hybrider Natur vorliegt. So findet man hier und da in der zweiten Reifeteilung Chromosomen, die zwischen den beiden Schwesterplatten liegen, wie dies für Speziesbastarde so äusserst charakteristisch ist. So habe ich in einem besonders klaren Fall in den beiden Schwesterplatten 19 Chromosomen zählen können und zwischen diesen an der Zellengrenze 3 eliminierte Chromosomen. In einem anderen Testis kommen wieder Platten der zweiten Reifeteilung mit der diploiden Chromosomenzahl nicht selten vor. Dieselben sind durch Vereinigung der Interkinesekerne entstanden, wovon man sich durch ein eingehendes Studium der Zysten mit Zellen mit diploider Chromosomenzahl leicht überzeugen kann. Sehr oft ist die Vereinigung der Kerne eine unvollständige und es entstehen allerhand unregelmässige Teilungen als eine Folge der Tetrapolarität. Die Zentrosomen sind nämlich in der Regel vier. Die Chromosomenzahlen 40 und 42 konnten in einigen Fällen exakt festgestellt werden. Auch die Querschnitte von Spermienbündel zeigen öfter, dass ein oder ein paar Spermienköpfe weit grösser als die übrigen sind. Es handelt sich offenbar um diploide Spermien.

Die Chromosomenzahl der Mischlinge ist von besonderem Interesse. Während beide Elternformen die Zahl 21 haben, ist für mehrere Mischlinge die Zahl *20* charakteristisch. Es hat also eine Fusion zwischen Chromosomen stattgefunden und zwar zwischen einem der grössten und

einem mittelgrossen Chromosom. In zwei von den Mischlingen Pernå × Schlesien ist diese Fusion in der Regel so vollständig, dass nur ein etwas dünneres Ende des Chromosoms dessen Charakter eines Fusionschromosoms verrät. In den beiden Kreuzungen Pojo × Böhmen kann man dagegen die beiden Elemente des Fusionschromosoms deutlich unterscheiden, und in einigen Fällen ist es sogar Geschmacksache, ob man die Chromosomenzahl als 20 oder 21 angeben will. Zwei Mischlinge der Kreuzung Pernå × Schlesien zeigen überhaupt keine Fusion, sondern haben ganz klar 21 Chromosomen wie die Elternformen. Ich gebe hier die von mir vorgenommenen Zählungen der Chromosomen in den sechs Testes in tabellarischer Form wieder.

Anzahl der Spermatozyten					Chromosomenzahlen			
					19	*20*	*21*	*22*
Pernå × Schlesien	1.	Spermatozyten	I		1	178		
		»	II			68	1	
» »	2.	»	I			110	1	
		»	II			78		
» »	3.	»	I				103	1
		»	II				53	
» »	4.	»	I				63	
		»	II				55	
Pojo × Böhmen	1.	Spermatozyten	I		1	48		
		»	II			6	8	2
» »	2.	»	I			94	3	
		»	II			45		

Da das Fusionschromosom von besonderem Interesse ist und während der Spermatogenese am besten erforscht werden kann, habe ich es in den verschiedenen Stadien der Spermatogenese eingehend studiert. In den Spermatogonien ist es nicht möglich gewesen ein grösseres Chromosom zu entdecken, geschweige denn die Chromosomenzahl exakt festzustellen. In dem Pachytänstadium kann man das Fusionschromosom auch nicht identifizieren. Erst in der Diakinese ist es in den meisten Kernen klar erkennbar. Meistens hat es die Form eines länglichen Körpers, in dem man in der Regel, jedoch nicht immer, eine leichte Einkerbung entdecken kann. Es kann aber auch einen grossen ovalen Körper bilden und nimmt zuweilen die Form eines Dreiecks an. Durch seine erhebliche Grösse ist es immer identifizierbar. In der ersten Reifeteilung ist das Fusionschromosom in den Mischlingen Pernå × Schlesien

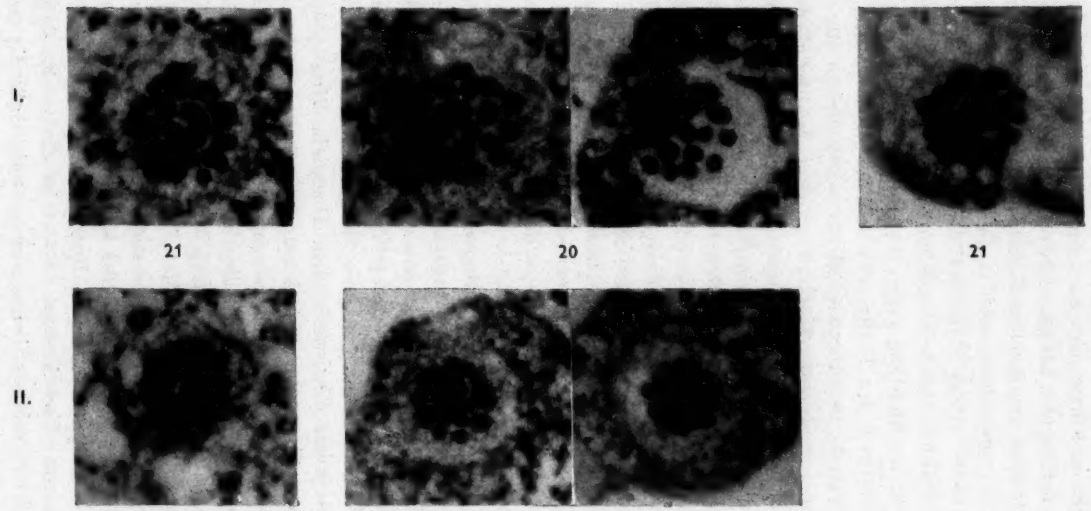


Fig. 13. Platten der Spermatozyten I in der oberen und der Spermatozyten II in der unteren Reihe; links von *germanica*-Raupen aus Böhmen, rechts von *fennica*-Raupen aus Pernå, in der Mitte von Mischlingen zwischen *fennica* und *germanica*.

1 und 2 immer sehr leicht von den übrigen Chromosomen zu unterscheiden. Es bildet im Gegensatz zu diesen, die oval oder rund sind, stets einen länglichen Körper, der an dem einen Ende etwas dünner ist. Dieser dünnere Teil ist das kleinere Chromosom, das mit dem grossen fusioniert ist. In ganz vereinzelten Fällen ist die Grenze zwischen den verschmolzenen Chromosomen auch in diesen Testes durch eine leichte Einkerbung markiert. In der Platte liegt das Fusionschromosom mit der Längsachse in der Äquatorialebene, nur ausnahmsweise sieht man es mit der Längsachse in der Richtung gegen den Pol. Es befindet sich weiter öfter an der Peripherie der Platte, viel seltener in der Mitte. In der Anaphase teilen sich die fusionierten Chromosomen gleichzeitig und man kann keine Spur von irgendwelchen Anomalien bei der Teilung entdecken. Alle konjugierten Chromosomen trennen sich ungefähr gleichzeitig, das fusionierte kommt jedoch meistens ein wenig später als die übrigen zum Pole. In der zweiten Reifeteilung liegen die Verhältnisse ganz ähnlich, nur sind sie der geringen Grösse der Chromosomen zufolge weniger deutlich. In einigen Zysten kann die Furche zwischen den fusionierten Chromosomen in gewissen Zellen deutlicher hervortreten. Sie kann jedoch auch vollständig fehlen, was wenigstens in einigen Fällen darauf beruht, dass das Chromosom schief steht und das grössere das kleinere bedeckt. Auch in der zweiten Reifeteilung ist die Anaphase ganz regelrecht.

Man findet also in der Spermatogenese keine besonderen Merkmale, die für eine stattgefundene Translokation in der einen der Elternrassen und eine hierdurch hervorgerufene anomale Bindung der Chromosomen in der Meiose des Mischlings sprechen würden. Ich habe mich ganz besonders bemüht in der Diakinese Ringe zu finden und habe auch ein paar solche in jedem Testis gefunden. Da solche Ringe jedoch auch in den Hoden der reinen Formen gleich oft und sogar öfter entdeckt werden, so kann das Vorkommen vereinzelter Ringe beim Mischling durchaus nicht als einen Beweis für eine Translokation betrachtet werden. Man müsste die Ringe regelmässig nachweisen können, wenn sie das Resultat einer Translokation wären.

b. *Oogenese.* — Von der Oogenese habe ich ganz besonders die zweite Reifeteilung untersucht, weil hier keine Verkettung der Chromosomen vorkommt und man dazu die Chromosomenzahl des zukünftigen Eies gewissermassen näher kommt. Zwar ist die zweite Reifeteilung im allgemeinen ihrer geringen Grösse wegen weniger günstig und erwies sich auch in diesem Fall undankbar. Die wirklich guten und eindeutigen Platten sind deshalb nicht zahlreich. Sehr oft bleibt man im Zweifel,

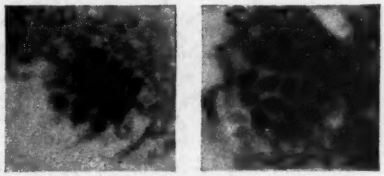
welche Zahl die richtige ist. Ich habe nur die klarsten Fälle, in denen man mit grosser Sicherheit die Zahl hat feststellen können, hier als Brüche zusammengestellt und zwar so, dass die äussere Platte im Zähler, die innere im Nenner steht, und ein Fragezeichen bedeutet, dass die Zahl nicht exakt bestimmt werden konnte.

Chromosomenzahlen der Oozytenplatten II.

	20/20	21/20	20/21	21/21	?/18	?/19	?/20	20/?	?/21	21/?	N
fennica×*germanica*	6	4			1	1	8	5	2	2	29
germanica×*fennica*	8	1	1	1			3	3	5		22

Wie aus der Zusammenstellung hervorgeht, sind die Platten mit 20 Chromosomen entschieden in der Mehrzahl. Besonders in der inneren Platte ist die Zahl 21 selten; sie kommt überhaupt nur zweimal vor. Die Fig. 14 zeigt uns photographische Aufnahmen von einer inneren und einer äusseren Platte mit 20 Chromosomen.

Es ist also klar, dass bei diesem Mischling in den Reifeteilungen beider Geschlechter eine Neigung zur Fusion von zwei Chromosomen vorliegt, die jedoch nicht bei allen Individuen manifestiert wird.



20 20

Fig. 14. *fennica* × *germanica*. Oozytenplatten II. Links eine äussere, rechts eine innere Platte aus zwei verschiedenen Eiern.

c. *Blastoderm.* — Es wäre selbstverständlich von grossem Interesse zu erfahren, ob dieselbe Neigung zur Fusion zweier Chromosomen, die in den Reifeteilungen beider Geschlechter zum Ausdruck kommt, schon in den somatischen Zellen vorhanden ist, oder ob die Verhältnisse dort anders liegen. Leider sind gute Präparate von den Mitosen während der Blastodermbildung nicht leicht zu erhalten. Erstens ist es schwer das richtige Stadium zu treffen, und zweitens macht die Fixierung Schwierigkeiten. Nur in drei Eiern ist es mir gelungen gute Blastodermmitosen zu finden. In der Fig. 15 sind sechs solche Mitoseplatten mit grösster Genauigkeit wiedergegeben. Die Zahl der Chromosomen in diesen beträgt 40, 41 und 42. Diese Zahlen sind nicht etwa für besondere Eier charakteristisch, sondern in demselben Ei kommen Platten mit verschiedenen Zahlen vor. In zahlreichen Fällen ist es nicht möglich zu entscheiden, ob zwei Chromosomen mit den Enden dicht aneinander liegen, oder ob es sich um ein einziges Chromosom handelt. Ich habe deshalb um möglichst unparteiisch bei der Zählung zu sein,

die Platten zuerst genau gezeichnet und sodann erst an der fertigen Zeichnung die Chromosomen gezählt. Nach den erreichten Resultaten scheint es mir kaum möglich zu entscheiden, ob tatsächlich auch in den

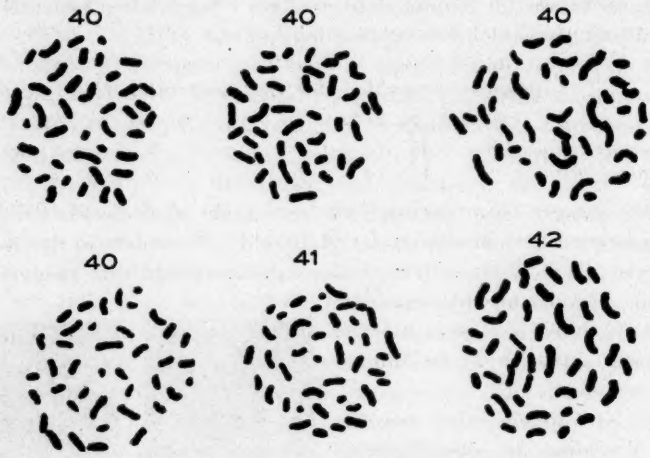


Fig. 15. *fennica* × *germanica*. Blastodermmitosenplatten.

somatischen Zellen eine Fusionstendenz vorliegt, oder ob nur eine zufällige Agglutination hier und da in den Zellen zum Vorschein kommt. Für eine definitive Lösung dieser Frage ist ein grösseres und besser fixiertes Material nötig.

C. D. VINULA GERMANICA ♀ × VINULA V. FENNICA ♂.

Diese Kreuzung wurde von mir im Jahre 1922 zwischen einem von Herrn KURT JOHN in Sachsen-Altenburg erhaltenes Weibchen und einem Männchen aus Kyrkslätt in Südfinnland ausgeführt. Leider misslang auch diese Zucht, so dass mein Material sehr dürftig ist.

a. *Spermatogenese.* — Nur ein Hoden wurde untersucht. In zwei Spermatogonien gelang es die Chromosomenzahl exakt festzustellen. Sie war dieselbe wie bei den Elternarten, nämlich 42. In sämtlichen von mir studierten Metaphaseplatten dieses Hodens konnte auch nur die normale haploide Zahl 21 gefunden werden. Es handelte sich um 65 Platten der ersten und 55 der zweiten Reifeteilung. Die Spermatogenese unterscheidet sich also bei dem von mir untersuchten Individuum dieses Mischlings in keiner Weise von derjenigen der Elternformen.

b. *Oogenese.* — Auch hier habe ich mein Interesse auf die zweite

Reifeteilung konzentriert, und auch hier können wir dieselbe Neigung zur Fusion zweier Chromosomen konstatieren wie bei der reziproken Kreuzung. Man vergleiche die Zusammenstellung auf Seite 235. Die Zahl 20 ist die häufigste; in 8 Eiern konnte diese Zahl in beiden Platten festgestellt werden und nur einmal die Zahl 21 in beiden. Ausserdem kommt die Zahl 21 in 5 Eiern in der inneren Platte vor; sie ist also hier etwas häufiger als bei dem reziproken Mischling, wenn man nach den wenigen Fällen überhaupt das Recht zu vergleichen hat.

D. RÜCKKREUZUNGEN MIT DEN REZIPROKEN BASTARDEN ZWISCHEN FENNICA UND GERMANICA.

Bei der Rückkreuzung des Bastards *fennica* × *germanica* wurden Weibchen aus der Zucht Pernå × Schlesien mit Männchen aus Pernå gekreuzt. Da von den vier untersuchten Männchen des in Rede stehenden Bastards zwei in den Reifeteilungen 20 Chromosomen besassen, zwei dagegen 21 und bei den Weibchen allerhand Kombinationen von 20 und 21 Chromosomen konstatiert wurden (vgl. S. 235), so ist es nicht möglich zu wissen, zu welchem Typus die bei den Rückkreuzungen benutzten Individuen gehörten. Es scheint mir deshalb nicht angebracht genaue Angaben über die von mir vorgenommenen Chromosomenzählungen hier mitzuteilen. Ich kann mich damit begnügen nur kurz zu erwähnen, dass in zwei von mir genau untersuchten Hoden dieses Rückkreuzungsbastards lauter Spermatozyten mit 21 Chromosomen zu finden waren und dies sowohl in der ersten als in der zweiten Reifeteilung.

Es wurden auch eine Anzahl Eier dieser Rückkreuzung untersucht. Leider war die Fixierung nicht gut gelungen, so dass die Anzahl der Chromosomen nur in wenigen Eiern exakt festgestellt werden konnte. In der Mehrzahl aller untersuchten Eier war sie jedenfalls 21, aber in einigen Eiern war es nicht möglich zu entscheiden, ob 20 oder 21 Chromosomen vorhanden waren, so dass vielleicht dennoch eine gewisse Neigung zur Fusion vorlag. Das Vorkommen einer solchen Tendenz kann jedoch nicht als bewiesen angesehen werden, da eine Agglutination der Chromosomen bei der ersten Reifeteilung in den Oozyten der meisten Lepidopteren beobachtet werden kann.

Es wurden ausserdem verschiedene andere Rückkreuzungen, z. B. solche mit der Vaterart *germanica* ausgeführt. Auch kompliziertere Verbindungen wurden erreicht. Das Material aus diesen Kreuzungen ist jedoch so spärlich, dass es sich kaum lohnt das Resultat der Chromosomenzählungen hier wiederzugeben. Kurz möge nur erwähnt wer-

den, dass in den untersuchten Testes die Zahl 21 immer gefunden wurde und dass nur ganz vereinzelte Eier mit 20 Chromosomen entdeckt wurden. Die Zahl 21 war also auch in den Eiern die absolut dominierende.

a. *Parthenogenese.* — In zahlreichen Eiern aus der Rückkreuzung *fennica* (Kyrkslätt) ♀ × [*germanica* (Sachsen-Altenburg) ♀ × *fennica* (Kyrkslätt) ♂] ♂ wurden Verhältnisse entdeckt, die auf eine beginnende Parthenogenese hindeuten. Es wurden zunächst deutliche Polkerne festgestellt, die sich teils jeder für sich, teils miteinander vereinigt, in verschiedenen Phasen der Mitose befanden. Die Reifeteilungen hatten also offenbar stattgefunden. Im Ei wurden aber ausserdem eine wechselnde Anzahl Kerne in Mitose entdeckt, von denen die meisten die haploide Chromosomenzahl aufwiesen. Es kamen jedoch auch solche mit der diploiden Zahl vor, die aber immer in der Minderzahl blieben. Die untersuchten Eier zeigten untereinander verglichen erhebliche Unterschiede. Obgleich die Eier zu verschiedenen Zeiten nach der Ablage fixiert wurden — die ältesten waren 3 Stunden alt — so konnte in keinem weder ein Spermatozoon noch ein Spermakern entdeckt werden. In normalen Eiern ist dies sonst immer der Fall. In Eiern von einem Alter von 2—3 Stunden pflegt die Vereinigung des Sperma- und des Eikerns stattgefunden zu haben, oder das Ei bereitet sich auf die erste Furchungsteilung. In den von mir untersuchten Eiern kam schon eine grosse Anzahl Kerne und Mitosen vor, woraus wir zu dem Schluss berechtigt sind, dass die Entwicklung schon in den Follikeln vor der Ablage der Eier angefangen hatte. Sowohl in den Polkernen wie in den Deszendenten des Eikerns kommt es zur Bildung von Restitutionskernen, wodurch die diploide Chromosomenzahl in einigen Mitosen ihre Erklärung findet.

Es scheint, als ob die Neigung zu einer parthenogenetischen Entwicklung durch die Bastardnatur der Eier angeregt würde. Die sicher unbefruchteten Eier des Bastards *albanica* × *germanica* zeigen, wie wir erfahren werden, auch eine beginnende Entwicklung (vgl. S. 239). Bei Pflanzen ist es keine seltene Erscheinung, dass die Bastardierung eine Parthenogenese auslöst, und sie kann hier sogar zur Entwicklung keim- und entwicklungsfähiger Samen führen.

E. D. VINULA ALBANICA ♀ × VINULA GERMANICA ♂.

Von dieser Kreuzung standen mir nur 9 Eier von einem unbefruchteten Weibchen zur Verfügung. Das Weibchen war ziemlich alt, als ich es tötete, die Eier aus dem Abdomen herauspräparierte und fixierte.

Es waren die letzten Eier und sie waren, wie gesagt, sicher unbefruchtet.

a. *Oogenese.* — Drei von den Eiern sind völlig normal, indem sie wie unbefruchtete Eier überhaupt nur eine regelrechte Metaphase der ersten Reifeteilung enthalten. In zwei von diesen kann die Chromosomenzahl 19 ganz exakt festgestellt werden. Eine solche Platte ist in der Fig. 16 abgebildet.

Die sechs übrigen Eier zeigen dagegen höchst eigentümliche Ver-

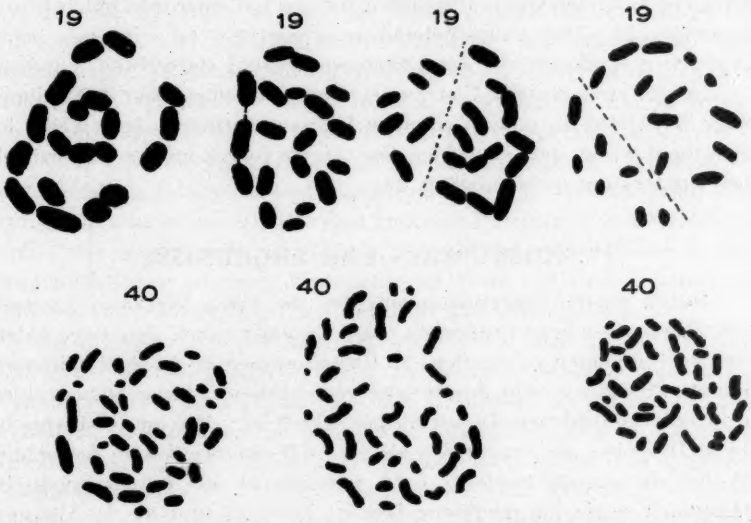


Fig. 16. *albanica* × *germanica.* Obere Reihe: erste Platte links, eine Oozytenplatte I, die übrigen, Oozytenplatten II. Untere Reihe: Blastomerenmitosen in einem unbefruchteten Ei.

hältnisse. In vier von diesen kann eine Platte mit der haploiden Chromosomenzahl 19 nachgewiesen werden; von diesen sind drei in der Fig. 16 wiedergegeben. Die Platten haben ganz den Charakter von Reifeteilungen, denn die Chromosomen sind dick und kurz. Ausserdem kommen aber in den Eiern eine grosse Anzahl Mitosen vor, in denen man teils die diploide Chromosomenzahl 40 feststellen kann, teils polyploide Zahlen von hoher Ordnung vermuten muss. In diesen Platten haben die Chromosomen den somatischen Typus und sind länglich und schlank. Die haploide Platte fasse ich als die Oozytenplatte II oder den sich teilenden reifen Eikern auf. Die erste Reifeteilung hat also stattgefunden, und die Oozyte II, resp. der reife Eikern, befindet sich

in der Metaphase, die offenbar eine Ruhepause durchmacht, während der erste Polkern sich sofort geteilt hat. Die Tochterkerne des Polkerns haben sich sodann miteinander vereinigt und auf solche Weise die diploide Chromosomenzahl restituiert. Bei einer Wiederholung dieser Kernverschmelzungen sind die polyploiden Kerne entstanden. Es liegt aber auch die Möglichkeit vor, dass die erste Teilung des Polkerns eine Restitutionsteilung war, was eher das zahlreiche Vorkommen von diploiden Kernen erklären würde. Für die erste Annahme spricht dagegen das Auftreten von multipolaren Mitosen mit einer sehr hohen Chromosomenzahl. Das wahrscheinlichste scheint mir zu sein, dass beide Typen von Verdoppelung der Chromosomenzahl stattgefunden haben.

In den zwei übrigen Eiern war es nicht möglich eine Reifeteilung unter den vielen di- und polyploiden Mitosen zu finden. Ich zweifle jedoch nicht daran, dass auch hier eine Oozyte vorgekommen ist, obgleich ihre Entdeckung nicht möglich war.

IV. DISKUSSION DER ERGEBNISSE.

Durch unsere Untersuchungen über die Arten *Dicranura erminea* und *D. vinula* wurde festgestellt, dass *erminea* (nach den zwei untersuchten Individuen zu urteilen) *28* Chromosomen in den Reifeteilungen besitzt, wogegen *vinula* durch sehr verschiedene Chromosomenzahlen in ihren verschiedenen Rassen ausgezeichnet ist. Die nordafrikanische Rasse *Delavoiei*, die vermutlich als eine selbständige Art zu betrachten ist, hat die grösste haploide Zahl, nämlich *31*, die mitteleuropäische *germanica* sowie die nordische *fennica* bloss *21* und die in Albanien lebende Form *albanica* die niedrigste Zahl *20*, trotzdem sie der *Delavoiei* mit der grössten Chromosomenzahl phänisch am ähnlichsten ist.

Es fragt sich, kann man in diesen Zahlen eine Entwicklungsserie erblicken? Es ist bekanntlich der Versuch öfter gemacht worden, die Chromosomenzahlen der Arten einer Gattung von einander herzuleiten, und in einigen Fällen hat man geglaubt eine bestimmte Entwicklungstendenz zur Verringerung der Chromosomenzahl, in anderen dagegen umgekehrt zur Vermehrung derselben feststellen zu können. Der Verfasser hat in anderem Zusammenhang anlässlich einer Untersuchung über die Chromosomenverhältnisse der Tagschmetterlinge (FEDERLEY, 1938) die Ansicht ausgesprochen, dass die Fragestellung eine unrichtige und veraltete ist. Es braucht überhaupt nicht eine Tendenz in der einen oder der anderen Richtung vorzuliegen. Die heutige Genetik rechnet nicht mit bestimmten Entwicklungstendenzen, sie kennt nur Mutationen

als Schritte der Evolution, und diese Mutationen sind — wenn wir die von JOLLOS nachgewiesenen als Ausnahmen betrachten — richtungslos. Wir kennen auch zahlreiche Mutationen, die Veränderungen im Chromosomensatz einer Art hervorrufen und diese beschränken sich nicht nur auf die Grösse und Form der Chromosomen, sondern beeinflussen auch die Chromosomenzahl. Ich verweise auf meine (FEDERLEY, 1938) gegebene Zusammenstellung solcher Fälle.

In welchem deszendenztheoretischen Verhältnis die hier behandelten *Dicranura*-Formen zu einander stehen scheint mir auf Grund der Chromosomen nicht möglich zu entscheiden. Wir können nur feststellen, dass die bei den Lepidopteren und vielen anderen Insekten öfter beobachtete Tatsache, *je kleiner die Chromosomenzahl um so grösser die Chromosomen*, auch in unserem Falle klar zum Ausdruck kommt. Die Form *albanica* mit der Zahl 20 hat die robustesten Chromosomen und ihr folgen *germanica* und *fennica* mit ihren 21 Chromosomen. Die 28 Chromosomen von *erminea* sind bedeutend kleiner, und die kleinsten treffen wir bei *Delavoiei*, die 31 hat. Der Gedanke, dass Fusionen oder Fragmentationen zu einer Verteilung der Gene auf eine verschiedene Zahl von Chromosomen geführt hat, liegt hier bestechend nahe.

Zu derselben Schlussfolgerung werden wir auch durch das Verhalten der Chromosomen bei den Mischlingen zwischen den untersuchten Formen gezwungen. Bei der Kreuzung der beiden Formen *germanica* und *fennica*, beide mit der haploiden Zahl 21, entstehen Bastarde mit der erwarteten Zahl 21, aber auch solche, die in beiden Geschlechtern nur 20 Chromosomen haben. Die Kreuzung zwischen *albanica* ($n = 20$) und *germanica* ($n = 21$) ergibt wiederum ein Weibchen mit 19 Chromosomen in der ersten Reifeteilung. Schon in meiner ersten etwas mehr eingehenden Mitteilung über die Kreuzungen *fennica* × *germanica* (FEDERLEY, 1937) wurde die Möglichkeit angedeutet, dass hier vielleicht eine Translokation vorlag. HANS BAUER (1941) sieht es fast als bewiesen an, dass hier infolge einer Translokation ein quadrivalentes Chromosom gebildet worden ist, muss jedoch zugeben, dass in diesem Falle die zweite Reifeteilung die für die Elternarten charakteristische Zahl 21 aufweisen müsste. Dies ist nun nicht der Fall, und dieser Umstand spricht meines Erachtens entschieden gegen die Hypothese von einer Translokation. Ich habe mir trotzdem grosse Mühe gegeben die Diakinesen der Bastarde mit 20 Chromosomen zu studieren um eventuell solche Ringe zu entdecken wie sie z. B. PELLEW and SANSOME (1931) und HÅKANSSON (1936) bei *Pisum*-Bastarden gefunden haben. Leider sind die Lepidopteren für solche Studien wegen ih-

rer kurzen, oft fast kugeligen Chromosomen schlecht geeignet. Man findet jedoch dann und wann bei Arten mit grossen Chromosomen, wie sie bei *vinula* vorkommen, deutliche Kreuze und Ringe. Bei den Bastarden habe ich jedoch durchaus nicht mehr Ringe als bei den reinen Formen finden können. Ringe sind überhaupt eine sehr seltene Erscheinung. Für die Annahme, dass die Verringerung der Chromosomenzahl die Folge einer Translokation und der Bildung eines quadrivalenten Chromosoms wäre, kann man in den zytologischen Verhältnissen des Bastards keine Beweise oder Anhaltspunkte finden. Mir scheint die von mir verfochtene Ansicht, dass eine durch die Kreuzung hervorgerufene Neukombination von Genen bei dem Bastard eine Fusion von zwei Chromosomen ausgelöst hat, wahrscheinlicher.

Diese hypothetische Annahme gewinnt an Wahrscheinlichkeit, wenn wir die eben besprochenen Verhältnisse mit den in der Kreuzung *Delavoiei* × *fennica* gefundenen vergleichen. Hier verbinden sich sämtliche 31 Chromosomen von *Delavoiei* mit den 21 Chromosomen von *fennica*, so dass der Bastard in der grossen Mehrzahl seiner Spermatozyten nur 21 Chromosomen aufweist. Es kommen zwar auch Spermatozyten mit einer grösseren Chromosomenzahl vor, aber wie die Tabelle S. 228 zeigt, bilden die Zellen mit der Zahl 21 eine absolute Majorität. Es ist klar, dass eine grosse Übereinstimmung in bezug auf die Gene und ihre Anordnung in den Chromosomen bei beiden Formen vorliegt, sonst wäre eine Konjugation, wie sie hier in dem Bastard stattfindet, überhaupt nicht denkbar. HANS BAUER (1941) schliesst sich in seiner Kritik über meine Auffassung, dass die Gene ihre Verteilung auf die Chromosomen und damit auch die Chromosomenzahl selber bestimmen, SEILER (1922) an und zitiert SEILER: »In den Chromosomenbastarden stellt sich bei der Conjugation eine Garnitur auf die andere um» . . . »für die Dauer der Conjugation und noch etwas darüber hinaus». Was heisst aber, dass die Chromosomen »sich umstellen»? In unserem Falle vereinen sich die 31 *Delavoiei*-Chromosomen mit einander, d. h. sie gehen Fusionen ein, indem sie mit den 21 *fennica*-Chromosomen konjugieren. Man könnte sich jedoch das »Sich-Umstellen» der Chromosomen ebenso gut so vorstellen, dass die 21 *fennica*-Chromosomen sich in 31 Einheiten aufteilten und mit den 31 Chromosomen von *Delavoiei* konjugierten. Übrigens was bedingt den Unterschied in der Chromosomenzahl von *fennica* und *Delavoiei*? Es scheint mir kaum möglich diesen Unterschied anders als einen genischen aufzufassen, und wenn zwei Formen einander so nahe stehen, dass sie ebenso leicht wie Individuen derselben Art mit einander gekreuzt werden

können, trotzdem sie eine sehr verschiedene Chromosomenzahl besitzen, so liegt wohl die Annahme, dass dieser Unterschied in der Chromosomenzahl durch eine Mutation entstanden ist, ausserordentlich nahe.

Auch der Bastard *erminea* (n = 28) × *vinula* (n = 21) bietet in bezug auf die Chromosomenzahl vieles von Interesse. Unter der Voraussetzung, dass die Chromosomen der Elternarten ihre Individualität und Autonomie beibehalten, hätte man bei totaler Affinität zwischen den 21 *vinula*-Chromosomen und einer entsprechenden Anzahl *erminea*-Chromosomen beim Bastard 28 Chromosomen erwartet und von diesen 21 bivalente und 7 univalente. Bei fehlender Affinität wäre das zu erwartende Resultat die Summe der haploiden Chromosomenzahl der beiden Elternarten oder 49 gewesen. Bei partieller oder wechselnder Affinität wären wiederum alle Zahlen zwischen 29 und 48 zu finden gewesen. Wie schon in der Beschreibung der Chromosomenverhältnisse des betreffenden Bastards (S. 220) hervorgehoben wurde, ist dies nun nicht der Fall. In dem Hoden A fanden wir eine Zahlenreihe von 27—51, in der nur die Zahlen 28 und 46—50 fehlen. Die beiden äussersten Zahlen 27 und 51 liegen also ausserhalb der zu erwartenden Zahlenreihe. Der Hoden B zeigt noch viel überraschendere Verhältnisse, indem er Zahlen von 35 bis 64 aufweist. Hier müssen also alle Chromosomenzahlen über 49 als eine Folge einer stattgefundenen Fragmentierung der Chromosomen zustande gekommen sein. Es liegt also klar auf der Hand, dass die Chromosomen der Elternarten im Bastard ihre Individualität aufgegeben und andere Verbände gebildet haben.

In diesem Zusammenhang möchte ich auch daran erinnern, dass die beiden Arten *bifida* und *furcula* der naheverwandten Gattung *Cerura*, welche Arten sich sehr leicht miteinander kreuzen lassen, so verschiedene Chromosomenzahlen wie 49 und 29 haben. In dem Bastard »stellen sich» jedoch die 49 *bifida*-Chromosomen nicht so »um», dass sie mit den 29 *furcula*-Chromosomen konjugieren würden, sondern man findet in den Reifeteilungen eine Serie Chromosomenzahlen, die zwischen 53 und 70 liegt. In diesem Bastard bewahren also die Chromosomen allem Anschein nach ihre Individualität und die Zahl der konjugierenden Chromosomen ist in den verschiedenen Spermatozyten eine sehr verschiedene (vgl. FEDERLEY, 1939).

BAUER hat in seiner Kritik die Chromosomenzahlen in den Blastodermzellen des Bastards als ausschlaggebend für die Auffassung, ob eine Fusion oder eine Translokation vorliege, bezeichnet. Von dem Bastard *Delavoiei* × *fennica* besitze ich leider keine fixierten Blastodermzellen, was sehr zu bedauern ist, da hier der Unterschied zwischen den Chro-

mosomenzahlen ein erheblicher ist. Dagegen habe ich Blastodermzellen des Bastards *fennica* × *germanica* fixiert. Es handelt sich also um die Frage, ob schon die somatischen Zellen eine Fusion von zwei Paaren Chromosomen aufweisen. Wie ich (FEDERLEY, 1937) schon hervorgehoben habe, ist eine so kleine Differenz wie 2 Chromosomen in einer Mitoseplatte von 40 resp. 42 Chromosomen sehr schwer exakt festzustellen. Es ist mir jedoch gelungen einige verhältnismässig gut fixierte und demzufolge klare und eindeutige Platten zu erhalten. Diese sind von mir mit grosser Sorgfalt gezeichnet worden (Fig. 15), und erst nach der Fertigstellung der Zeichnung wurden die Chromosomen an dieser gezählt, ein Verfahren, das grösste mögliche Objektivität bei Bestimmung der Chromosomenzahl garantiert. Es stellte sich nun heraus, dass die Zahlen 40, 41 und 42 alle drei vertreten waren und sich ganz zufallsmässig auf die drei Eier verteilten. Dieses Resultat ist also nicht ausschlaggebend und spricht meines Erachtens eher dafür, dass eine Fusionstendenz vorkommt, die sich jedoch nicht immer manifestiert. Dies ist ja auch der Fall in der zweiten Reifeteilung, wo die Bindung viel schwächer als in der ersten ist.

Ich bin mir übrigens dessen klar bewusst, dass alles Reden von Fusionen und Fragmentationen heutzutage als ganz unzeitgemäss angesehen wird. Besonders die *Drosophila*-Forscher — und sie bilden ja die Mehrzahl der Genetiker — wollen von solchen diffusen Prozessen nichts wissen. *Drosophila* ist zwar in genetischer Hinsicht und Leistungsfähigkeit ein ganz besonderes Tier und wird wohl ihre Stellung als *Primus inter pares* noch lange behalten, aber die an *Drosophila* gewonnenen Erfahrungen in allen Einzelheiten auf andere Tiere zu übertragen, scheint mir nicht richtig. Wenn es tatsächlich an *Drosophila* geglückt ist nachzuweisen, dass keine Fusionen von ganzen Chromosomen stattfinden und dass Translokationen nur mit Bruchstücken von Chromosomen geschehen, so dürfen diese Beobachtungen nicht verallgemeinert werden, und wenn weiter Chromosomenfragmente ohne das Kinetochor bei *Drosophila* immer eliminiert werden und zu Grunde gehen, so braucht dies durchaus nicht bei anderen Tieren und bei Pflanzen der Fall zu sein. Es ist sogar ganz klar, dass das Kinetochor bei anderen Insekten durchaus keine so wichtige Rolle wie bei der Taufliege spielt. Ich möchte hier einige Beobachtungen und in der letzten Zeit ausgeführte Experimente mit diesem Gesichtspunkt vor Augen in Erinnerung bringen.

Zunächst verdient die von SCHRADER schon 1935 gemachte Beobachtung an dem langen X-Chromosom von *Protenor belfragei* Erwähnung,

weil sie zeigt, dass sogar dasselbe Chromosom in zwei aufeinander folgende Teilungen ein ganz verschiedenes Verhalten aufweisen kann. In der Äquationsteilung liegt das X-Chromosom rechtwinklig gegen die Spindelachse und die beiden Chromatiden gehen parallel auseinander. Ein Kinetochor steht also nicht zu entdecken. In der Reduktionsteilung liegt das X-Chromosom dagegen parallel zur Spindelachse und ist an den Enden durch eine feine Faser mit den Polen verbunden. Dass diese Faser die Rolle einer Zugfaser spielt geht dadurch hervor, dass das Ende des X-Chromosoms öfter zugespitzt und ausgezogen erscheint.

In dem Verhalten des X-Chromosoms bei *Protenor* sind es zwei Umstände, die uns ganz besonders interessieren. Der erste ist, dass eine Teilung des Chromosoms ohne ein feststellbares Kinetochor stattfindet, der andere dass in der folgenden Teilung, die zwar eine Reduktionsteilung ist, ein Kinetochor vorhanden zu sein scheint. Diese Tatsachen erwecken Zweifel daran, dass das Kinetochor oder das Zentromer, nach der DARLINGTONschen Terminologie, wirklich als eine permanente Organelle existiert, wie die *Drosophila*-Forscher und mit ihnen eine grosse Anzahl der Zytologen auf botanischem Gebiet meinen, und dass wenn ein solches tatsächlich nachgewiesen werden kann, ob es nicht de novo bei jeder Teilung entsteht. Jedenfalls ist es klar, dass die Verhältnisse in bezug auf das Kinetochor bei verschiedenen Tieren verschieden sind. Die konstante Lage des Kinetochors — oder sagen wir lieber die konstante Befestigungsstelle der »Zugfaser» — bei den *Drosophila*-Arten, den Orthopteren und besonders bei den Liliaceen (vgl. z. B. DARLINGTON, 1940) kann wohl kaum geleugnet werden. Aber deshalb braucht diese Anordnung nicht bei allen Organismen durchgeführt zu sein. Bei den Schmetterlingen deutet nichts darauf, dass bei ihnen ein Kinetochor vorkäme. Dies kommt besonders klar zum Vorschein in der Oogenese, wo die Chromosomen grösser und vor allem länger als in der Spermatogenese sind. In den Eiern kann man deutlich die erste Reifeteilung verfolgen und man beobachtet hier, wie sich die deutlich sichtbaren Konjuganten parallel voneinander entfernen, ohne dass eine Biegung oder Knickung wahrnehmbar würde. Chiasmata kommen überhaupt nicht in der Oogenese der Lepidopteren vor, und in den kugeligen Chromosomen der Spermatogenese ist dies noch weniger der Fall. Dagegen sind interzonale Fasern oft ganz deutlich vorhanden, wie ich schon in der Beschreibung S. 213 besonders hervorgehoben habe. Sie erinnern ganz an die von SCHRADER (1932, S. 528) bei *Syromastes* abgebildeten. Vermutlich spielt der Stemmkörper BELARS

bei den Lepidopteren eine nicht unwichtige Rolle bei der Trennung der Chromosomen, denn er tritt hier öfter auffallend klar hervor.

Eine Untersuchung, die uns noch stärker davon überzeugt, dass das Kinetochor keine permanente Organelle ist, haben neuerdings SALLY HUGHES-SCHRADER und HANS RIS (1941) ausgeführt. Sie behandelten Cocciden mit Röntgenstrahlen und erreichten hierdurch Fragmentierung der Chromosomen. Diese Fragmente verhielten sich ganz wie gewöhnliche Chromosomen während der Mitosen und in der Meiose. Die Spiralisation, die Pro-, Meta- und Anaphase der Fragmente waren nicht von den entsprechenden Stadien der ganzen Chromosomen zu unterscheiden. Keine Elimination, nicht einmal ein Nachhinken der Fragmente, konnte beobachtet werden. Die Anaphasen sind sogar wunderschön, und die einander entsprechenden Fragmente können in den Tochterplatten mit absoluter Sicherheit identifiziert werden. Diese Versuche wurden mit *Steatococcus tuberculatus* vorgenommen, einer Art, deren haploide Männchen nur 2 Chromosomen haben. Durch die Behandlung wurden nicht weniger als 24 Fragmente erhalten, und diese verhielten sich wie normale Chromosomen durch zahlreiche Zellgenerationen.

Die von SALLY HUGHES-SCHRADER und HANS RIS erzielten Resultate sind für das Problem von der Entstehung der verschiedenen Chromosomenzahlen bei den Lepidopteren von grossem Interesse. Sie beweisen, dass es nicht a priori unmöglich ist, dass durch Fragmentierung neue Chromosomenrassen entstehen können, wie FEDERLEY (1938) vorausgesetzt hat. Wir kennen tatsächlich Arten, die in verschiedenen Rassen auftreten, und diese Rassen unterscheiden sich von einander ausschliesslich in bezug auf die Chromosomenzahl. Die verschiedenen Rassen enthalten ein oder mehrere Sammelchromosomen. Ob diese durch Fusionen entstanden sind, oder ob sie die ursprünglichen sind und durch Zerfall die Rassen mit zahlreicheren Chromosomen ergeben haben, ist nicht leicht zu entscheiden. *Leptidia sinapis* ist eine solche Art mit einer wechselnden Zahl von Chromosomen, wie FEDERLEY (1938) für die nordischen Formen und LORKOVIĆ (1941) für die südeuropäischen Formen nachgewiesen haben. SEILER (1925) hat bei *Phragmatobia fuliginosa* zwei Rassen gefunden, von denen die eine westlich von der Elbe verbreitet ist und 28 Chromosomen hat, die andere östlich von der Elbelinie vorkommt und 29 Chromosomen besitzt. Die erstgenannte hat ein langes Chromosom, das bei der letztgenannten auf zwei selbständige Chromosomen verteilt ist. Einen besonderen Fall haben wir bei *Lymantria monacha*, bei welcher gewisse Chromosomen

ganz regelmässig sowohl während der Spermatogenese als der Oogenese eine Fusion eingehen und also ein Sammelchromosom bilden um in den ersten somatischen Zellen wieder einer Fragmentierung unterworfen zu sein und zur ursprünglichen Chromosomenzahl zurückzukehren. Hier haben wir also eine normale temporäre Fusion gewisser Chromosomen in dem Zyklus (SEILER und HANIEL, 1921).

LORKOVIĆ leugnet die Annahme, dass die grossen Chromosomen durch eine Fusion zweier oder mehrerer Chromosomen entstanden wären und ist der Ansicht, dass Fusionen in der Entstehung der Chromosomenzahlen überhaupt keine Rolle gespielt haben. Hierzu ist er offenbar durch die von BAUER vertretene Auffassung inspiriert worden. Er hat aber selber das Vorkommen von Sammelchromosomen bei *Leptidia sinapsis* beschrieben und abgebildet. Er hat sogar 2—4 Ansatzstellen der Zugfasern in diesen Sammelchromosomen feststellen können. Viele von den von LORKOVIĆ abgebildeten grossen Chromosomen zeigen durch ihre eckige Form, dass sie den Namen Sammelchromosomen verdienen. Damit ist durchaus nicht gesagt, dass alle grossen Chromosomen Sammelchromosomen wären. FEDERLEY (1938) hat sogar ausdrücklich davor gewarnt, jedes grosse Chromosom als das Produkt von einer Fusion mehrerer Chromosomen aufzufassen. Es gibt aber zahlreiche Indizien dafür, dass viele der grossen Chromosomen ihre Entstehung einer Vereinigung kleinerer Chromosomen zu verdanken haben. Belege für die Richtigkeit dieser Auffassung wurden soeben angeführt.

Der Genetiker denkt in Genen, der Zytologe in Chromosomen. Der Zytogenetiker sollte sowohl in Genen als Chromosomen denken, eine Aufgabe, die jedoch mit Schwierigkeiten verbunden ist, denn es fällt sich nicht leicht den Konflikt zwischen der Suprematie der Gene und der Hierarchie der Chromosomen zu lösen.

Schon 1932 hat DARLINGTON diese Frage in einem sehr lesenswerten Aufsatz behandelt. Er gibt in diesem mehrere Beispiele von einer Kontrolle der Gene über die Chromosomen, scheint jedoch der Ansicht zu sein, dass diese Kontrolle sich zur Form, Grösse und Bewegung der Chromosomen beschränkt. Die Anzahl der Chromosomen dürfte nach ihm nicht durch Gene determiniert werden. Bei Bastarden zwischen Eltern mit verschiedenen Chromosomen sowohl in bezug auf Form als Anzahl spricht er von strukturbedingten Verhältnissen. DARLINGTON kommt schliesslich zu der Schlussfolgerung, dass es genbedingte und

strukturbedingte Chromosomenmerkmale bei den Bastarden gibt. Damit scheint mir nicht viel gewonnen zu sein, denn was verstehen wir unter Struktur — in erster Linie handelt es sich wohl um die Chromosomen. Zweifelsohne gibt es Bastarde, in denen die Chromosomen ihre Form, Grösse und Anzahl hartnäckig beibehalten, trotzdem sie in ein fremdes Plasma geraten sind. Aber es kommen auch Fälle vor, in denen die Chromosomen der Bastarde Fusionen oder Fragmentationen unterworfen sind, und diese sind wohl meistens auf die Wirkung bestimmter Gene oder richtiger Genkombinationen zurückzuführen.

Ich habe mich mehrmals und entschieden für die Oberhand der Gene über die Chromosomen ausgesprochen, was den Zytologen nicht gefallen hat, denn diese wollen für die Chromosomen die Suprematie behalten. In den Chromosomen sehe ich nur phänische Merkmale, die wie alle solche von den Genen und den Umweltbedingungen im weitesten Sinne beeinflusst werden. Dadurch dass die Gene ihren Sitz in den Chromosomen haben, bilden diese zwar eine ganz besondere Art von Merkmalen und unterscheiden sich dadurch von allen übrigen. Gegen diese Auffassung hat, wie gesagt, BAUER (1941) Stellung genommen und meint, man könnte die von mir bei den *Dicranura*-Bastarden festgestellten Tatsachen anders deuten. Was ich z. B. bei dem Bastard *fennica* × *germanica* eine Fusion von zwei Chromosomen genannt habe, will er als eine Translokation ansehen, die zur Bildung einer Vierergruppe geführt hat und auf solche Weise die Chromosomenzahl mit einer Einheit reduziert hat. In der Kreuzung *Delavoiei* × *fennica* sollen wiederum nach BAUER mehrere solche Vierergruppen und sogar Sechsergruppen und Gruppen von noch höherer Valenz entstehen. Wie die meisten *Drosophila*-Forscher stellt sich BAUER auf dem Standpunkt, dass eine Fusion zwischen zwei unbeschädigte ganze Chromosomen ein Ding der Unmöglichkeit wäre. Nur wenn ein Endstück fehlt, kann eine Fusion stattfinden, aber man nennt sie dann Translokation. Das Wort Fusion sollte man überhaupt vermeiden und ebenso den Terminus Fragmentation, denn in der Evolution spielen sie keine Rolle. Von den Fragmenten kann sich nach BAUER bloss dasjenige mit dem Kinetochor beibehalten, während die übrigen eliminiert werden. Ich habe mich jedoch nicht durch die Argumentierung von BAUER überzeugen lassen, und es scheint mir, als ob die Zytologen die Sache zu einseitig betrachten.

Wenn zwei Arten eine verschiedene Chromosomenzahl besitzen, so liegt selbstverständlich ein struktureller Unterschied vor. Dieser strukturelle Unterschied kann ein sehr erheblicher sein, wie z. B. zwischen *Cerura furcula* mit 29 und *C. bifida* mit 49 Chromosomen und

trotzdem ist die genische Differenz eine relativ geringe, denn die Ähnlichkeit der beiden Arten ist sehr gross, die Kreuzung derselben sehr leicht und der Bastard gedeiht vorzüglich. In anderen Fällen kann eine morphologisch identische »Struktur» zwischen zwei Arten vorliegen, und sie sind trotzdem sehr verschieden und lassen sich nicht miteinander kreuzen. Immer muss jedoch der strukturelle Unterschied, möge er gross oder klein sein, auf eine genische Differenz zurückgeführt werden. Wenn zwei verschiedene Chromosomensätze durch Kreuzung in einer Zygote vereint werden, so ist es selbstverständlich die neue Kombination von Genen, die die Struktur des Karyotypus, d. h. die Chromosomen, ihre Zahl, Form und Grösse determiniert. Zwar werden wohl die Zygote und die somatischen Zellen des Bastards überhaupt in der Regel die Summe der Chromosomen der Elternarten aufweisen, weil die Gene anfangs während der Entwicklung inaktiv sind. Die Chromosomen behalten also anfangs ihre Autonomie bei, aber in der Meiose verändern sich die Verhältnisse. Jetzt werden die Gene aktiv und bewirken die Umgruppierung der Gene in andere Verbände und die Autonomie der Chromosomen geht verloren. Die Individualität der Chromosomen ist also keine absolute, sondern eine relative. Sie wird durch den Einfluss anderer Genome verändert, was zur Entstehung ganz neuer Karyotypen führen kann. Eine solche Umgruppierung der Gene bedeutet ja schliesslich keine Veränderung des eigentlichen Genotypus. Wenn die im Bastard entstandene Neukombination von Genen eine harmonische und lebensfähige ist, so wird sie stabilisiert und ein in morphologischer Hinsicht neuer Karyotypus ist geschaffen.

Dass Mutationen in derselben Art wie Bastardierungen wirken können, ist an und für sich nichts befremdendes. Wir können wohl deshalb auch mit der Möglichkeit rechnen, dass durch Mutationen neue Karyotypen und vor allem auch Veränderungen in der Chromosomenzahl hervorgerufen werden.

Unter Umständen kann eine Veränderung der Chromosomenzahl auch mit einer wirklichen Zunahme der Chromosomen nebst deren Genen und nicht nur mit einer verschiedenartigen Verteilung des ursprünglichen Genbestandes verbunden sein. Das beweisen die zahlreichen Beispiele von einer sehr verschiedenen Zahl von Mikro- und Geschlechtschromosomen bei verschiedenen Insektenarten. Ich erinnere zunächst an den alten bekannten Fall von überzähligen Chromosomen bei *Metapodius* (WILSON, 1910), den von REUTER (1930) beschriebenen Fall bei *Alydus* und die neuerdings bei *Gryllotalpa africana* durch ASANA, MAKINO und NIIYAMA (1940) und bei *Gryllotalpa vulgaris* (STEOPOE,

1939) entdeckten Verhältnisse in bezug auf die wechselnde Zahl von X-Chromosomen sowie schliesslich an die Untersuchungen über die X-Chromosomen bei *Cimex lectularius*. Nach SLACK (1939) und DARLINGTON (1940) kann die Anzahl der X-Chromosomen von 4—16 variieren. Offenbar handelt es sich hier um Chromosomen, die verhältnismässig sehr arm an Genen sind, jedenfalls an solchen Genen, die eine vitale Bedeutung haben. Sonst wäre eine so beträchtliche Zunahme eines Chromosoms an der Zahl nicht möglich.

V. ZUSAMMENFASSUNG.

Es wurden die Chromosomenzahlen folgender Arten und Rassen der Gattung *Dicranura* bestimmt: *erminea*, n = 28; *vinula germanica*, n = 21; *vinula* var. *fennica*, n = 21; *vinula albanica*, n = 20; *vinula* var. *Delavoiei*, n = 31.

Diese Arten und Rassen lassen sich leicht miteinander kreuzen. Bei allen Mischlingen findet eine äusserlich ganz normale Synapsis, von einem vollständig typischen Pachytänstadium gefolgt, statt. Die Konjugation ist jedoch so schwach, dass in den Reifeteilungen einiger Mischlinge eine grosse Anzahl, ja sogar lauter univalente Chromosomen zu finden sind. Es kommt auch vor, dass der Mischling in den Reifeteilungen eine grössere Anzahl Chromosomen als die Summe der haploiden Chromosomenzahlen der Elternarten aufweist, was wohl als Folge einer Fragmentation der Chromosomen zu erklären ist. Aber das Umgekehrte, eine Verminderung der Chromosomenzahl zu oder unter der geringeren der Chromosomenzahlen der Elternarten, ist auch mit Sicherheit festgestellt worden. Diese Verminderung wird vermutlich durch Fusionen von zwei oder mehreren Chromosomen verursacht.

Die bei den Mischlingen beobachteten Chromosomenzahlen sind die folgenden:

erminea × *vinula*	Hoden	A	27—51	M = 36,98
» × »	»	B	35—64	M = 45,71
erminea × (*erminea* × *vinula*)	»	1	35—47	M = 41,88
» × » × »	»	2	32—46	M = 39,54
Delavoiei × *fennica*	»	1	20—37	M = 23,78
» × »	»	2	21—24	M = 21,41
» × »	Oozyten	I	19—26	M = 21,56
fennica × *germanica*	4 Hoden		20	
» × »	2 »		21	
albanica × *germanica*	Oozyten	I	19	

In den Eiern einiger Bastarde wurde beginnende parthenogenetische Entwicklung festgestellt.

Die Frage, ob Fragmentationen und Fusionen von ganzen Chromosomen vorkommen können, wird diskutiert und vom Verf. bejaht.

ERKLÄRUNG DER ABBILDUNGEN.

Sämtliche Abbildungen sind mit Hilfe des monoobjektiv-binokularen Mikroskopes und der ABBE'schen Zeichenkamera vom Verf. direkt auf dem Arbeitstisch gezeichnet. Optik: Apochrom. Objektiv, homogene Ölimmersion, Brennweite 1,5 (Einzelvergrösserung 120), Kompensationsokular 20 ×. Die Vergrösserung beträgt also etwa 2500.

Die Mikrophotographien der Figg. 4 *a*, 11, 13 sind mit dem »Phoku» von ZEISS aufgenommen. Es wurde das Objektiv mit 120-maliger Einzelvergrösserung benutzt. Die erhaltenen Negative wurden bei der Darstellung der Positive dreimal vergrössert. Die Vergrösserung ist etwas geringer als diejenige der Zeichnungen.

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THE PIGMENT CONTENT OF POLYPLOID PLANTS

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AT a comparison of the different qualities of diploids and autopolyploids it is necessary to pay attention to, on one side, the primary cellular change and, on the other side, the more secondary change in anatomical organisation and developmental rhythm. The chief cellular change that occurs at chromosome doubling is an increase in nuclear and cellular volume. This involves an increase in quantity of the different components of the cell, as for instance, the nucleolus and the cytoplasm. The size of the chromosomes, on the other hand, has in certain cases been shown to diminish as their number increases. The chloroplasts show a certain agreement with the chromosomes, their number is increased in proportion to the increase in cell volume, while their size is unchanged or possibly somewhat diminished (KOSTOFF, 1938).

The anatomical and physiological response may be referred to the increase in cellular volume, all organs growing thicker and more robust. Longer time and a greater bulk of material is needed for building up a larger cell. This may explain the slower mitotic rhythm and the more sluggish organic growth of the polyploids.

In order to get a picture of the chlorophyll content of an autopolyploid as compared with its original form histological methods may be used for an estimation of the thickness of the chlorophyll-containing layers and their concentration of chloroplasts. RASMUSSON and LEVAN (unpublished) found this method unfavourable in such a plant as the sugar beet, in which the histological differences between diploids and tetraploids are insignificant and hard to grasp.

Another method and one that may more readily yield reliable results is the preparation of leaf extracts, the pigment content of which may be determined. Such analyses of autoploid materials were first made by KOSTOFF (l. c.), who found that the chlorophyll content per square unit in 2x, 4x and 8x *Nicotiana* increased with increasing ploidy. Later on GYÖRFFY (1941) and PIRSCHLE (1941, 1942) made several such determinations with the use of SEYBOLD-EGLE's chromatographic method. In GYÖRFFY's material only little regularity was found: on 25

occasions of analysis of 8 species the tetraploids had higher chlorophyll content than the diploids in 12 cases counted on green matter, in 17 cases counted on dry matter, and in 20 cases counted on unit area (l. c., Table 11, p. 375). PIRSCHLE found in three genera a consistently falling chlorophyll content with increasing ploidy counted on the weight unit of green matter, but increasing chlorophyll content per unit of surface.

The results are presented below of a preliminary examination by a simple photometric method of the pigment content in a rather rich material of diploids and polyploids which were available in Svalöf last summer. I wish to express my gratitude to my colleagues for generously putting material at my disposition. My sincere thanks are also due to Dr. H. BURSTRÖM, Ultuna, for his kind help in the working out of a suitable method of chlorophyll analysis, to Dr. H. KAJMER, Svalöf, for valuable advice as to the photometric work throughout the investigation, and to Dr. O. TEDIN, Svalöf, for a statistical scrutiny of the results. Most of the photometer determinations were made by Mr. G. MICHANEK, Lund.

I. METHOD.

The sampling always involves a great source of error in this kind of work. In order to reduce these errors as far as possible, those samples which were to be compared were always taken simultaneously and were afterwards treated in exactly the same manner. Comparable parts of the leaves were selected: in Gramineae leaves were taken from the middle of the straw, in the beets well-developed but non-aging leaves were taken, etc. When possible (*Secale*, *Hordeum*, *Trifolium*, *Galeopsis*, *Beta*, *Linum*) leaf samples were collected from 25 different individuals chosen at random in the whole field plot. Corresponding parts of the leaves were then sectioned and mixed. The petioles and the leaf tips as well as diseased parts were sorted out. Out of the cut and carefully mixed leaf sample three samples were simultaneously taken with 5 or 10 g in each (10 g were taken of the more heterogeneous materials as, for instance, *Beta*): *1.* for chlorophyll analysis, *2.* for determination of the water content, and *3.* for measurement of the square surface.

The chlorophyll sample was ground with quartz sand and extracted quantitatively with pure acetone. From the filtered raw extract of chlorophyll a series of dilutions was prepared suitable for anlysis in a

HELLIGE Photometer, in which the light extinction of the extract was compared with the extinction of pure acetone. The apparatus is graduated in direct concentration values (from 0 to 0,75); so the relation between different extinctions indicates the relation of the concentrations. Determinations were made in $\lambda = 660$ mμ, which absorbs mainly the green components of the pigment, and in $\lambda = 500$ mμ, which absorbs the yellow components. These two values, however, showed only very slight variations in their relation to each other, and therefore in chapter II only the values of $\lambda = 660$ will be recorded.

In order to make clear the course followed in the analyses the detailed procedure in one instance is given here: *Mangel beets*, analysed $^{15}/_{7}$, 1 $^{1}/_{2}$ month after the sowing and $^{1}/_{2}$ month after the thinning. The plants had 6—9 leaves, the roots weighed about 1 g. 10 g of leaves were extracted to 100 cc. From this extract 4 dilutions were made: 2, 4, 8 and 16 %. The following extinction values were obtained (for the sake of simplicity the hundredths, read on the apparatus, are given as whole numbers throughout this report):

Concentration		0	2	4	8	16 %
2x	λ 660:	8	16	24	38	51
	λ 500:	4	9	13	19	33
4x	λ 660:	8	14	21	33	55
	λ 500:	4	8	11	18	32

Thus each chlorophyll determination gave a dilution curve, which furnished a good control. If the curve as in the above case was even and plausible, a representative chlorophyll value was selected, viz. the extinction of 0,2 g green matter per 100 cc. After subtracting the zero value we obtain in the above instance λ 660: 8—6 (here as elsewhere in this paper the lower chromosome number is mentioned first), and λ 500: 5—4. The tetraploids showed lower chlorophyll content counted on green matter.

The chlorophyll content estimated on dry matter might have been calculated from the former values, but it was considered an additional control to determine this value from the curve independently of the green matter value. The extinction curves were therefore reconstructed with the dry matter weights on the x-axis. As a representative value the extinction of 0,05 g dry matter per 100 cc was selected. In the above instance the water content was 90,6—92,6 %, the dry matter chlorophyll

value being λ 660: 20,6—21,6, λ 500: 11,0—11,8. Thus, by this operation the tetraploid curve has been moved above the diploid curve.

The square surface of the leaves was calculated on the basis of contour drawings of 10 g fresh leaves. As the extinction curve in the range used is straight-lined, the extinction values could be used as absolute chlorophyll values, and they could consequently be extended to the chlorophyll content of 1 dm^2 leaf substance. In the above instance 10 g of leaves had a superficial area of 2,6682—2,4409 dm^2. Since 0,2 g green matter showed the extinction 8—6, the extinction per dm^2 was 149,9—122,9.

In order to make it possible to convert the extinction values obtained in our photometer into the more generally used PULFRICH units, Dr. KAJMER made a series of parallel chlorophyll determinations in our apparatus and in a PULFRICH photometer. The relation between the two apparatuses was very nearly rectilinear. In fact, our values had to be multiplied by 1,4 below 40 and 1,3 above 40 in order to be changed into PULFRICH values.

In order to establish a further characteristic of our photometer a dilution series of the chlorophyll standard recommended by GUTHRIE (1928) was tested. We found that absolute chlorophyll weights (mg/100 cc) could be obtained from our extinction values by multiplying by a factor 0,06296 (λ 660) and 0,10264 (λ 500). These calculations were carried out, but the variation of the chlorophyll contents was found to be somewhat wider than could be expected; so, in order to avoid a possible error, I prefer to describe the chlorophyll contents as the direct extinction values. The absolute chlorophyll values calculated by means of GUTHRIE's chlorophyll standard were of the following general character. Per green matter: *Allium* 1 ‰, *Beta* 2—4 ‰, *Populus*, *Galeopsis* 5—9 ‰, *Rumex obtusifolius* 11 ‰; per dry matter: *Allium*, *Linum* 10 ‰, *Beta* 20 ‰, *Galeopsis*, *Phleum*, *Secale*, *Hordeum* 30 ‰; mg chlorophyll per dm^2: 5—20.

II. PIGMENT ANALYSES.

In this chapter a record is given case for case of the analyses performed. For each plant type examined the following values are given:

A: extinction of 0,2 g green matter;

B: ditto of 0,05 g dry matter;

C: ditto of 1 dm^2 leaf surface;

D: water content of the green matter:

E: the surface in dm^2 of 10 g green matter.

Within brackets the value is given of the higher ploid in percentage of the value of the lower ploid.

1. *Secale cereale* (x = 7). — $^2/_7$, 2x and 4x Steel rye. A: 27—19 (70,4); B: 21,8—19,9 (91,3); D: 70,1—76,3 (108,9).

2. *Hordeum vulgare* (x = 7). — $^3/_7$, 2x and 4x Opal B, 4x was lower and coarser, its green colour distinctly darker, A: 21—16 (76,2); B: 19,0—16,2 (85,3); D: 71,8—75,4 (105,0).

3. *Phleum pratense* (x = 7). — $^5/_9$—$^{11}/_9$. Four single greenhouse plants with chromosome numbers varying from 3x (»haploid») to 12x were studied, and also eight pairs of twins (6x »diploid» and 9x »triploid»), which had been cloned into 12 tufts each and were grown in the garden. The plants were studied during the second flowering. The four greenhouse plants had the following values (in the sequence 3x—4x—7x—12x): A: 24—24—18—24; B: 19,4—22,0—19,7—18,6; D: 68,0—74,5—78,3—74,6. The values are rather irregular, as might be expected from a study of single unrelated individuals.

The values of the eight twin pairs are collected in Table 1. It is seen from this table that the differences in pigment content between the 6x and 9x plants are slight. Calculated on green matter the 6x have

TABLE 1. *Different twin pairs of Phleum pratense.*

Twin pair No.	A		B		C		D		E	
	6x	9x	6x	9x	6x	9x	6x	9x	6x	9x
1	26	31(119,2)	25,0	28,2(112,8)	222	357(160,5)	68,8	72,0(104,7)	5,85	4,34 (74,3)
2	19	17 (89,5)	17,7	14,5 (81,9)	155	171(110,3)	72,5	66,5 (91,7)	6,12	4,97 (81,2)
3	22	21 (95,5)	17,9	19,5(108,9)	196	210(107,4)	68,5	73,0(106,6)	5,62	5,00 (88,9)
4	29	27 (93,1)	25,1	27,3(108,8)	266	226 (85,0)	71,0	75,3(106,1)	5,45	5,96(109,5)
5	22	21 (95,5)	18,3	18,8(102,7)	175	212(121,1)	69,3	71,5(103,2)	6,28	4,95 (78,8)
6	21	23(109,5)	15,8	20,0(126,6)	184	267(145,1)	64,7	70,0(108,2)	5,69	4,30 (75,5)
7	24	21 (87,5)	22,9	15,1 (65,9)	183	194(105,8)	73,5	62,9 (85,6)	6,56	5,43 (82,7)
8	22	21 (95,5)	16,7	18,6(111,4)	171	211(123,4)	66,0	71,2(107,9)	6,44	4,98 (77,4)

some preponderance, but calculated on dry matter and on superficial area the 9x plants predominate, depending on their higher water content and smaller surface. The twin pairs analysed were of a very varying type, all kinds occurring from plants with a few thick and rigid leaves with a blue-green colour to tufts with many thin and delicate light-green leaves. This may explain the great variation in the results. Different

morphological types within a species evidently respond differently in this respect to an augmentation in chromosome number.

4. *Allium nutans* (x = 8). — $^{9}/_{7}$. In this species all kinds of somatic numbers occur. Owing to the frequent occurrence of giant gametes the somatic numbers of each new generation may be increased by n or sometimes even by 2n units. For the examination of the chlorophyll content I selected four closely related plants, each representing one step in the chromosome augmentation: 2x (5039—1), in the progeny of which one 4x plant (5735—12) was present. In the progeny of this 4x plant there occurred both one 6x (1356—1) and one 8x plant (1356—4). A very striking scale of directed differences was found in the morphology of the leaves: 2x had narrow thin leaves with dimensions similar to a *Phleum* leaf, while 4x and 6x had broader and thicker *Narcissus*-like leaves, the 8x, finally, had almost half-round leaves of great stiffness. A histological examination of the leaves showed that the frequency of chloroplasts was especially high in the external cell-layers on both sides of the leaf, but diminished rapidly towards the centre, which in the thicker leaves was practically devoid of chlorophyll. Thus, lower extinctions could be expected in the polyploids. The following values, in fact, show this to be the case:

	A	B	C	D	E
2x:	11	; 13,7	; 262,4	; 82,8	; 2,10
4x:	6 (54,6);	11,0 (80,3);	175,6 (66,9);	87,5 (105,7);	1,71 (81,5)
6x:	4 (36,4);	10,2 (74,5);	166,6 (63,5);	89,2 (107,4);	1,20 (57,3)
8x:	3 (27,3);	8,1 (59,6);	162,6 (61,9);	89,4 (108,0);	0,92 (44,0)

5. *Allium Schoenoprasum* (x = 8). — $^{7}/_{8}$. In the progeny of the triploid F_1 cross between the Siberian chive (4x) and ordinary 2x chive there occurred both 2x and 4x plants. One such pair was chosen for analysis. On this occasion the flowering was already over, so the 2x plant was decidedly further advanced than the 4x, which was a darker green. In spite of this the 4x plant contained less pigment, even counted per dry matter. A: 6,4—5,4 (84,4); B: 11,0—7,9 (71,8); C: 87,5—95,6 (109,3); D: 85,5—82,8 (96,8); E: 3,66—2,83 (77,3). The area of the leaves was calculated on split leaves.

6. *Allium Cepa* × *fistulosum* (x = 8). — $^{10}/_{7}$, one primary hybrid (2x) and one amphidiploid (4x) were examined. A: 4,2—3,8 (90,5); 9,2—10,4 (113,0); D: 88,6—90,9 (102,6).

7. *Populus tremula* (x = 19). — $^{7}/_{8}$, one 2x and one 3x tree trans-

planted from the first locality of giant aspen at Lillö and cultured for several years alongside each other at Svalöf. A: 19—19 (100,0); B: 10,2—11,7 (114,7); C: 160,8—155,6 (96,8); D: 58,3—60,7 (104,1); E: 5,91—6,10 (103,3).

8. *Rumex* (x = 10). — 4 species with different chromosome numbers were selected for analysis by Dr. A. LÖVE, viz. *R. conglomeratus* (2x, No. 332), *obtusifolius* (4x, No. 315), *crispus* (6x, No. 309) and *aquatilis* (20x, No. 346). Although these are spontaneous polyploids differentiated in nature, and the differences between them can therefore

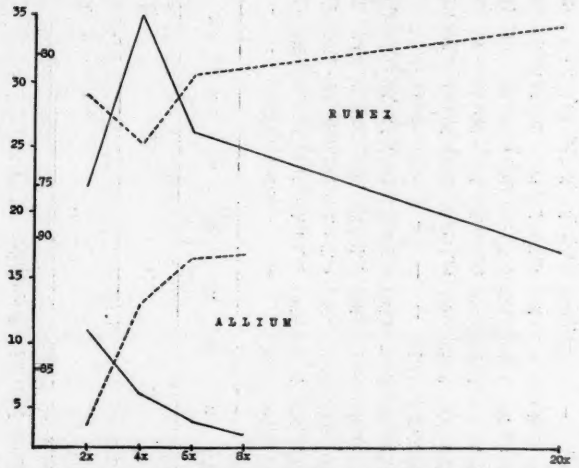

Fig. 1. The extinction per green matter (unbroken line) and water content (broken line) in different polyploids of *Rumex* and *Allium nutans*.

hardly be considered to be due, even mainly, to the differences in chromosome number, it was considered that it would be of some interest to cover such a wide range of somatic numbers as from 2n = 20 to 2n = 200. The following values were obtained:

	A	B	D
2x:	22	; 24,1	; 78,4
4x:	35 (159,1);	36,6 (151,9);	76,6 (97,7)
6x:	26 (118,2);	30,2 (125,3);	79,2 (101,0)
20x:	17 (77,3);	19,2 (79,7);	81,0 (103,3)

The extinction values indicate that the optimum of chlorophyll production is located in the region 4x—6x. This agrees with the results

TABLE 2. *Sugar beets and mangels.*

Date	Sugar beets										Mangels									
	A		B		C		D		E		A		B		C		D		E	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
1/7	6	6 (100,0)	15,7	15,4 (98,1)	—	— —	91,7	91,9(100,2)	—	— —	8	5 (62,5)	16,6	16,9(101,8)	—	—	91,8	93,3(101,6)	—	—
15/7	8	6 (75,0)	18,1	17,8 (98,3)	121	121 (99,9)	91,1	92,7(101,8)	3,30	2,48 (75,1)	8	6 (75,0)	20,6	21,3(103,4)	150	123 (82,0)	90,6	92,6(102,2)	2,67	2,44 (91,5)
3/8	9	6 (66,7)	18,2	14,9 (81,9)	157	112 (71,3)	89,6	89,8(100,2)	2,87	2,69 (93,5)	9	5 (55,6)	20,7	20,1 (97,1)	185	113 (61,2)	92,3	92,9(100,7)	2,43	2,21 (90,7)
17/8	12	10 (83,3)	21,8	22,1(101,4)	188	163 (86,3)	87,4	88,2(100,9)	3,19	3,08 (96,6)	11	10 (90,9)	21,0	24,6(117,1)	208	177 (85,1)	88,1	89,5(101,6)	2,64	2,82(106,8)
2/9	11	12 (109,1)	20,7	22,6(109,2)	164	192(117,4)	86,7	87,9(102,0)	3,36	3,12 (93,0)	13	13 (100,0)	23,8	27,7(116,4)	191	245(128,2)	87,4	90,4(103,4)	3,41	2,66 (78,0)
3/9	14	12 (85,7)	24,6	23,3 (94,7)	—	— —	86,2	87,7(101,7)	—	— —	15	11 (73,3)	22,8	28,2(123,7)	—	—	85,4	88,0(103,1)	—	—
16/9	18	17 (94,4)	21,0	25,6(121,9)	236	290(123,1)	84,3	87,2(103,4)	3,82	3,28 (85,8)	15	13 (86,7)	22,5	27,5(122,2)	191	210(110,2)	85,7	88,3(103,0)	3,93	3,09 (78,6)
1/10	12	9 (75,0)	20,4	18,7 (91,7)	173	140 (80,7)	85,3	86,7(101,6)	3,46	3,22 (93,0)	14	10 (71,4)	27,0	23,1 (85,6)	194	162 (83,7)	86,6	88,7(102,4)	3,61	3,08 (85,4)
19/10	8	7 (87,5)	15,2	11,9 (78,3)	147	103 (70,1)	83,5	83,8(100,4)	2,72	3,40(124,8)	6	5 (83,3)	13,1	13,4(102,3)	107	96 (89,7)	84,3	85,8(101,8)	2,81	2,61 (92,9)
30/10	6	6 (100,0)	11,5	13,0(113,0)	112	116(103,6)	86,9	86,7 (99,8)	2,68	2,58 (96,6)	6	8 (133,3)	14,3	14,7(102,8)	90	130(143,5)	85,9	85,7 (99,8)	3,32	3,08 (92,9)
16/11	11	8 (72,7)	11,5	12,7(110,4)	177	129 (73,1)	79,0	81,9(103,7)	3,12	3,10 (99,5)	12	—	14,2	—	—	—	81,7	—	—	—

of LÖVE (1942) in a polyploid series of another subgenus of *Rumex*. It should be noted that the chlorophyll values of *Rumex* behave differently from those of *Allium nutans*, in which the optimum was situated at the diploid number (Fig. 1).

9. *Beta vulgaris* (x = 9). — A field trial consisting of 9 repetitions of 3 rows 2x alternating with 3 rows 4x sugar beets (Hilleshög) and a similar arrangement of mangels (Barres) was sown on June 1st. The general result of this experiment, which was harvested at certain inter-

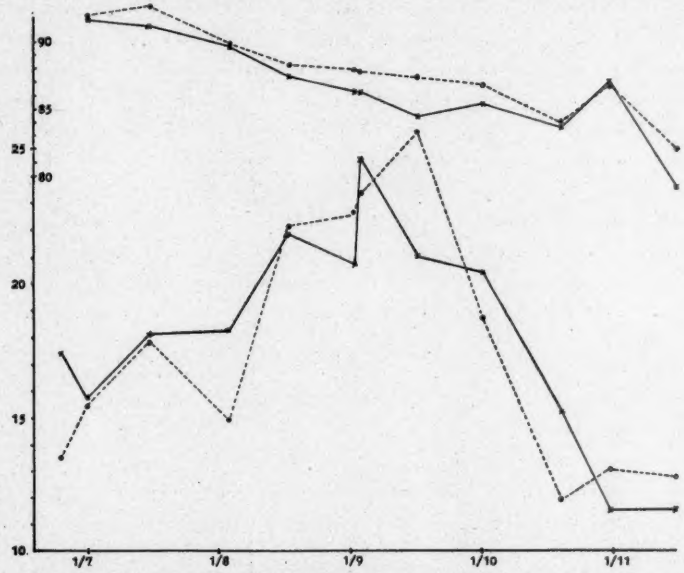

Fig. 2. The seasonal variation of the extinction per dry matter weight (lower curves) and of the water content (upper curves) of diploid (unbroken line) and tetraploid (broken line) sugar beets.

vals during the growth season, will be the subject of another paper. It may be mentioned here, though, that leaf samples for chlorophyll analysis were taken on 11 occasions between $^{1}/_{7}$ and $^{15}/_{11}$. It is quite evident that the sources of error are especially great in an experiment with such a highly varying material as commercial varieties of beets; so decisive results can hardly be expected. The experiment, however, may be regarded as a preliminary investigation of the behaviour of the pigment during the development and the ripening of the beet. The results are summarized in Table 2.

It is seen from this table that the 4x beets had on most occasions

lower chlorophyll content per fresh weight, but this difference is diminished if the dry weight values are considered. In the mangels the 4x leaves seem even to have more pigment. The values per unit area are rather erratic, 4x often having the lower value.

In spite of the frequent casual fluctuations a rather distinct picture may be got of the seasonal changes in pigment content of 2x and 4x beets. Fig. 2 shows the seasonal curve of the sugar beets, and conditions among the mangels are broadly speaking the same. The pigment content increases during the first part of the growth period, and the maximum is reached during August—September. After that the curves again go downwards. The tetraploids constantly have a somewhat higher water content, the water percentage falling during the summer from 92 to about 80 %. With a couple of exceptions, which may be considered as casual instances, the surface per unit fresh weight is smaller in the tetraploids.

10. *Melandrium rubrum* (x = 12). — $^{9}/_{7}$, 2 ♂ plants, one 2x (Mrs. D. LÖVE, No. 210) and one 4x (411) were tested. Even in the field it was clearly seen that the 2x plant was darker green than the 4x. The results confirmed this. A: 19—11 (57,9); B: 19,7—14,2 (72,1); D: 76,0—81,0 (106,6).

11. *Trifolium pratense* (x = 7). — $^{26}/_{6}$, plants belonging to a 2x—4x clone were examined. A: 18—18 (100,0); B: 22,3—21,9 (98,2); D: 80,3—80,8 (100,6).

12. *Trifolium hybridum* (x = 8). — $^{26}/_{6}$, 2x and 4x plants of a North-Swedish strain, overwintered in the field. A: 21,6—17,6 (81,5); B: 23,7—22,0 (92,8); D: 77,2—80,0 (103,6).

13. *Trifolium repens* (x = 8). — $^{11}/_{9}$, 4x and 8x plants from one vegetatively propagated plant. Leaves were collected from about 20 individuals of each kind. The plants were cloned during 1941 and were after that cultured in pots in the greenhouse: A: 25—17 (68,0); B: 29,0—27,7 (95,5); C: 208,4—185,2 (88,9); D: 79,0—84,3 (106,7); E: 6,00—4,59 (76,5).

14. *Linum usitatissimum* (x = 16). — Analyses were made three times during the summer, viz. $^{29}/_{6}$, $^{20}/_{7}$ and $^{10}/_{8}$, i. e. when the plants were respectively 6, 9 and 12 weeks old. Three strains were tested: 411/12 (originating from the commercial variety Herkules, a typical fibre flax), 413/14 (Concurrent, a fibre flax with certain properties of the oil flax) and 415/16 (Szekacs, a typical oil flax). Only the values of the analysis on the second occasion are given in Table 3. However, the relation between 2x and 4x was about the same on the other two occasions. Thus, the 4x flax showed rather constantly a lower chlorophyll content.

The seasonal curve of the pigment had a similar course to that in *Beta*, although in the case of *Linum* only three points on each curve were known. In the order 2x 411, 413, 415—4x 412, 414, 416 the dry matter

TABLE 3. *Linum*.

Strain	A		B		C		D		E	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
Herkules...	14	10 (71,4)	14,0	11,4 (81,4)	117	88 (75,5)	75,1	77,9 (103,7)	6,01	5,68 (94,6)
Concurrent	12	11 (91,7)	15,3	13,4 (87,6)	112	128 (114,2)	80,7	81,4 (100,9)	5,34	4,29 (80,3)
Szekacs ...	13	11 (84,6)	12,1	11,1 (91,7)	123	116 (94,2)	71,8	75,3 (104,9)	5,28	4,74 (89,8)

extinctions on the three occasions of analysis were as follows: $^{29}/_{6}$: 16,5, 15,5, 18,7—12,6, 14,5, 19,2; $^{20}/_{7}$: 14,0, 15,3, 12,1—11,4 13,4, 11,1; $^{10}/_{8}$: 9,2, 8,6, 9,6—8,0, 8,4, 7,9; thus, a mainly falling tendency.

15. *Galeopsis* (x = 8). — During the later half of July a number of analyses were made in different *Galeopsis* lines selected by Professor MÜNTZING. Among these were diploid and autotetraploid lines of *G. pubescens* (PA and PB and the tetraploid cross between them), further a diploid and an autotetraploid line of *G. speciosa* and the allotetraploid *pubescens* × *speciosa*, and in addition one 4x and one 8x line of *G. Tetrahit*. The results were as follows:

	A	B	C	D	E
PA	2x: 24	; 26,9	; 190,2	; 79,3	; 6,31
	4x: 19 (79,2)	; 26,3 (97,8)	; 180,3 (94,8)	; 83,6 (105,4)	; 5,27 (83,5)
PB	2x: 19	; 22,5	; 158,8	; 79,1	; 5,98
	4x: 15 (79,0)	; 20,1 (89,3)	; 136,2 (85,8)	; 81,3 (102,8)	; 5,51 (92,0)
PA × PB	4x: 17 (79,1)	; 22,1 (89,5)	; 144,6 (82,9)	; 81,0 (102,3)	; 5,88 (95,6)
spec.	2x: 22	; 28,6	; 195,5	; 82,0	; 5,63
	4x: 17 (77,3)	; 23,7 (82,9)	; 194,2 (99,3)	; 82,7 (100,9)	; 4,38 (77,8)
PA × *spec.*	4x: 20 (95,2)	; 25,9 (93,3)	; 199,9 (103,7)	; 83,7 (103,8)	; 5,00 (83,8)
Tetr.	4x: 25	; 26,5	; 266,7	; 76,7	; 4,69
	8x: 22 (88,0)	; 20,5 (77,4)	; 321,9 (120,7)	; 73,2 (95,4)	; 3,42 (72,9)

Thus, in this rather varying material the doubled types have constantly lower chlorophyll content.

III. CONCLUSIONS.

An examination was made of those analyses described in the preceding chapter in which a 2x or 4x form (the latter case in *Trifolium*

repens and *Galeopsis Tetrahit*) was directly comparable with a doubled form. Hence *Phleum*, *Populus*, *Rumex* and higher ploids of *Allium nutans* were excluded. The values of the two field trials of the plant development were represented by one mean for the sugar beets, one for the mangels and one for each of the three flax varieties. In the material thus obtained (Table 4) it was found that the doubled forms had

TABLE 4. *The values of the doubled forms in percentage of the values of the original forms.*

	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	Mean
A.....	2	—	1	2	4	4	3	1	—	1	—	—	—	—		79,0
B.....	—	—	—	2	1	3	2	4	4	—	1	1	—	—		90,1
C.....	—	—	1	—	1	—	2	3	2	—	1	1	—	1		94,9
D.....	—	—	—	—	—	—	—	—	2	10	6	—	—	—		102,9
E.....	—	—	—	1	3	3	2	2	1	—	—	—	—	—		81,3

constantly lower pigment content per green matter (A). The same was the case with the dry matter (with two exceptions though: *Allium Cepa* ×*fistulosum* and the mangels), but here the differences between the original and the doubled forms were considerably lower (B). Calculated per surface unit the results were rather discordant, in most cases (9 out of 12 cases) the pigment quantity of the doubled form was lower (C). The doubled forms always had higher water content (D) and smaller surface (E).

The question might now be raised whether the lower pigment content of the polyploids is due to a lower pigment content of each individual cell owing to a specific tendency of the polyploids to produce less pigment, or whether the cause should be sought in a changed anatomy of the polyploid leaves. The former alternative eludes judgment, because, even if it were possible to estimate the size and the number of the chloroplasts in perfectly comparable cells of 2x and 4x forms, it would still be uncertain that a fair comparison was being made. For instance, it is impossible to know whether such a factor as the pigment concentration of each chloroplast really is unchanged.

The latter alternative, however, may be tested in the present material. Certain of the plants examined give a distinct indication that the lower pigment content of the polyploids is due, partly in any case, to differences in the anatomy of the leaves. Especially the *Allium nutans* series is suggestive. It is a known fact that the leaves of the autopolyploids are thicker than those of the diploids. If the pigment

concentration decreases towards the centre of the leaf, and this situation is directly observed in *Allium nutans*, it must be expected that the polyploids should have a lower pigment content per weight unit than the diploids.

In other words, a positive correlation should be expected between the pigment quantity per green matter and the square leaf surface per weight unit. An examination was made in my material in order to investigate this question. The regression of the two qualities was determined in the different genera, each chromosome number being treated separately. In the only genus in which a comparatively great number of variates were present, viz. *Beta*, a strong positive regression was found. This regression had the same magnitude in the diploids (+ 5,55) as in the tetraploids (+ 5,62). The total regression *within* the ploids was + 5,61 and *between* the ploids + 5,91. The significance of all these values was very great. In the other genera the regression varied; on an average it was lower than in *Beta* and in all cases without any significance. In two cases (*Phleum* and *Galeopsis*) it was even weakly negative. Between the different genera an evident and strong positive regression was found of the same order of magnitude as in *Beta* (+ 4,31).

The correlation within the material as a whole was pronounced: + 0,79. Within each genus the result was varying (*Beta* + 0,68, *Linum* + 0,46, *Allium* + 0,39, *Phleum* — 0,10, *Galeopsis* — 0,11). The total correlation between the different genera was very strong: + 0,91. In order to examine whether all these correlations might be considered casual variations of the same correlation, a comparison was made between the correlations of *Beta* and *Phleum* with the aid of FISHER's z-analysis. The probability that they were all expressions of the same correlation was only 0,02—0,05.

Thus the pigment content has been shown to be highly dependent on the anatomy of the leaves: the larger the leaf surface per weight unit, the higher the pigment content. There may be a strong correlation within the plant types, as was the case in *Beta*. In such cases it may be expected that the tetraploids, which are characterized by a greater thickness of the leaves, also have a lower pigment content per weight unit. In other groups of plants this correlation seems to be less pronounced or even negative. The present investigation shows that it is not possible to frame a general rule for the behaviour of the pigment production of autopolyploids. The pigment content is dependent on the other changes which occur at the chromosome doubling. Greatest in importance among these are perhaps the changes in leaf thickness.

SUMMARY.

A simple photometric method for the determination of the pigment content in leaves was worked out. Pigment analyses were made in 11 genera, including diploids-tetraploids (*Secale*, *Hordeum*, *Allium*, *Beta*, *Melandrium*, *Trifolium*, *Linum*, *Galeopsis*), diploids-triploids (*Phleum*, *Populus*), and whole polyploid series (*Phleum*, *Allium*, *Rumex*). In *Beta* and, to a less extent, in *Linum* analyses were made on different occasions during the vegetation period. It was found that the diploids usually had higher pigment content per green weight than the polyploids. The same condition was found counted per dry matter, the difference being less, however. Counted per surface the results were more varying. The water content of the diploids was almost everywhere lower than that of the polyploids, and their surface per weight unit was constantly larger. The conclusion is drawn that one of the causes of the lower pigment content of the polyploids, and perhaps the most important one, is the greater thickness and consequently smaller surface of their leaves.

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GENETICAL AND PHYSIOLOGICAL STUDIES IN INTERSPECIFIC WHEAT CROSSES

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I. INTRODUCTION.

THE cross *Triticum vulgare* × *T. turgidum* has been studied by the author since 1933. In this year Prof. Å. ÅKERMAN handed over an F_2 of the winter wheat cross 0984 d (*vulg.*) × »Rivett's Bearded» (*turg.*) growing in the experimental fields of the Wheat and Oat Breeding Department of the Swedish Seed Association (Sveriges Utsädesförening), Svalöf. The parental variety 0984 d was selected at Svalöf out of the cross Sol II (Sun II) × Pansar I (Iron I) and is consequently a sister variety of 0986 Kron (Crown) and 0987 Stål (Steel), two Svalöf varieties well-known in South Swedish agriculture about 1930. The other parent is of English origin and is identical with the variety »Red Rivet» described by PERCIVAL (1921) in his wheat monograph.

In 1934 and subsequent years the material was enlarged by a great number of other cross combinations. The majority of these also belong to the species cross *T. vulgare* × *T. turgidum*, but also other wheat species have been included in the investigations. A total of 88 different varietal combinations have been studied. Only 66, however, are included in the present publication, all belonging to the winter form. The objects of the investigations are to inquire into (1) the segregation of some morphological characters, and (2) the inheritance of some physiological qualities, especially the resistance to cold and the connection between this inheritance and the chromosome number.

The experimental work has been carried out at Svalöf, and I wish to express my gratitude to the Institute of Genetics of the University of Lund and to the Swedish Seed Association for the support given to me in the shape of experimental ground and the loan of manual help. This support has been rendered to me on account of the interest and kindness shown in my investigations by Prof. H. NILSSON-EHLE, late head of both institutions, by Prof. A. MÜNTZING, present head of the Institute of Genetics, and by Prof. Å. ÅKERMAN, present head of the Swedish Seed Association and of its Wheat and Oat Breeding Department. To the

latter I also wish to express my special gratitude for his readiness to give me advice and suggestions, based upon his rich experience in wheat genetics and cold-resistance. To my colleagues at the Seed Association, Dr. A. LEVAN, Dr. O. TEDIN and Dr. GÖSTA ANDERSSON, I wish to acknowledge my indebtedness for their valuable assistance in different parts of the investigations. My wife, Mrs. MARGIT GRANHALL, has assisted me in a most deserving manner in the cytological investigations and also in other parts of the work. In connection with the field observations and the assorting of the harvested material I am indebted to Miss SIV LINDELL and to the many students who have assisted me during the different years. Finally I also desire to tender my gratitude to the Royal Physiographical Society of Lund and to the Jubilee Foundation of the Swedish Seed Association for grants made to me.

II. STUDIES ON SEED PRODUCTION AND MORPHOLOGY.

1. THE WHEAT SPECIES — A TAXONOMICAL SURVEY.

The genus *Triticum* includes a few wild and a multitude of more or less commonly cultivated forms. The present taxonomy of the genus is built up on 15 species, distributed over three main groups. These three groups were first established by SCHULZ (1913) on the strength of morphological similarities and differences. The grouping was confirmed by VAVILOV's (1914) observations on fungous resistance, ZADE's (1914) investigations on serum reactions, and TSCHERMAK's (1914) sterility studies in different cross combinations. In 1918 SAKAMURA presented the first correct chromosome counts, revealing the fact that the three groups formed a polyploid series with $2n = 14$, 28, and 42 respectively.

The list of species is in accordance with PERCIVAL (1921), with the addition of two later-described species, *T. persicum* (VAVILOV and JAKUSHKINA, 1925) and *T. Timopheevi* (ZHUKOVSKY, 1928; SVETOZAROVA, 1939). Another new species, *T. armeniacum* JAKUBZ., has been established by MAKUSHINA (1938) but its relations to *T. Timopheevi* are not quite clear (SVETOZAROVA, 1939).

In the present paper only one tetraploid species, *T. turgidum*, and three hexaploid ones, *T. vulgare*, *T. Spelta*, and *T. compactum*, are included in the investigations. As already mentioned, only winter forms are used. From PERCIVAL's (1921, pp. 154—158) descriptions of the wheat species the following extracts will be given in order to show the differences in some typical characters.

Group I »The small spelt wheats» Diploid (2n = 14)	Group II »The emmer wheats» Tetraploid (2n = 28)	Group III »The bread wheats» Hexaploid (2n = 42)
T. ægilopoides BAL. (wild form)	*T. dicoccoides* KÖRN. (wild form)	(no wild form known)
T. monococcum L.	*T. dicoccum* SCHÜBL.	*T. vulgare* HOST.
	T. persicum VAV.	*T. Spelta* L.
	T. turgidum L.	*T. compactum* HOST.
	T. durum DESF.	*T. sphærococcum* PERC.
	T. polonicum L.	
	T. pyramidale PERC.	
	T. orientale PERC.	
	T. Timopheevi ZHUK.	

(1) *T. turgidum* L. Rivet or Cone Wheat: *Straw*, tall, solid or hollow with thick walls. *Ear*, square in section or narrower across the face than the 2-rowed profile; bearded, heavy, often pendulous; rachis tough; awns stout, very scabrid from tip to base, frequently deciduous. *Empty glume*, short and broad, the outer face convex, keel prominent from tip to base; apical tooth stout, usually acute and curved; glumes more firmly attached to rachis than in *T. durum*.

(2) *T. vulgare* HOST. Bread Wheat: *Straw*, hollow with thin walls, though forms are occasionally found with solid upper internodes. *Ear*, square in section or more commonly broader across the face than the 2-rowed profile, bearded or beardless; the awns shorter than those of *T. durum* or *T. turgidum*; rachis tough. *Empty glume*, broad, the outer face convex, keeled from tip to base or in the upper half only; apical tooth in the bearded forms short, acute, or sometimes prolonged into an awn (1—4 cm. long), in the beardless forms usually short and blunter.

(3) *T. compactum* HOST. Club Wheat: *Straw*, hollow, very variable in length. *Ear*, bearded or beardless, short and dense; rachis tough. *Empty glume*, as in *T. vulgare*.

(4) *T. Spelta* L. Large Spelt or Dinkel: *Straw*, stout and hollow. *Ear*, very lax, bearded with short awns or beardless; rachis broad and stout, convex on one side, flat or concave on the other, fragile, breaking transversely below each spikelet. *Empty glume*, firm, with broad truncate apex; apical tooth short and blunt; prominent lateral nerve ending in a blunt projection.

Genetical surveys of interspecific crosses in wheat have been

published by KAJANUS (1927), WATKINS (1930), MATSUMURA (1939), and others. The genetics and cytology of the pentaploid wheat hybrids, which are of special interest in this connection, have been studied by a great number of investigators, among whom KIHARA and MATSUMURA (*T. Spelta* × *T. polonicum* and other crosses), SAX (*T. vulgare* × *T. durum*), A. A. and L. A. SAPEHIN (*T. vulgare* × *T. durum*), WATKINS (*T. vulgare* × *T. turgidum*), and THOMPSON (*T. vulgare* × *T. durum*, *T. vulgare* × *T. dicoccum*) are to be mentioned.

2. MATERIAL AND AIMS OF THE PRESENT INVESTIGATIONS.

The parental varieties used in the different crosses are gathered in Table 1, in which also their ear, glume, and grain characters are entered. In the last column their classification according to KÖRNICKE (cf. PERCIVAL, 1921) has been given.

In the lower half of the table a number of »*vulgare*»-, »*Spelta*»-, »*turgidum*»- and »*turgidum-contractum*-like» lines are collected. These lines — the numbers of which are all beginning with a »6» — have been extracted from F_4 or F_5 of the cross 0984 d *vulgare* × Red Rivet *turgidum* carried out in 1931. In order to obtain practically constant lines of different types out of this cross a repeated selection procedure has been performed. Thus vigorous and relatively fertile plants of hexaploid and tetraploid habitus were selected in F_2. Special attention was devoted to the ear type, glume shape, keel development, and cavity of the straw. In those F_3 families which showed good uniformity and similarity to different pure species the selection was repeated. In 1935 some of these F_4 lines were used as parents in different crosses. In 1936 F_5 lines, derived from new single plant selection, were also used in crosses and, moreover, in the physiological investigations, as will be reported in Chapter III: 3. The nomenclature of these lines has been built up upon the field numbers of 1936, the first year that the lines were tested in freezing trials. Line No. 9 in the plot-series 1936—366 has thus been named 6009, etc., and the whole series of lines from the cross No. *1* (0984 d × Red Rivet) has been called the »6000-series». The lines used for crosses as early as 1935 are usually given a compound number, e. g. 6009/11, which means that the line in question represents the F_4 plot from which the F_5 lines 6009, 6010, 6011 are derived (see also Table 17).

The »8000»-, »9000»-, and »0000-series» have been denominated in a corresponding way by their field numbers in 1938, 1939, and 1940 respectively, when they entered the freezing trials. A closer descrip-

TABLE 1. *The parental varieties and lines and their morphological characters.*

	Ear	Glumes	Grain	
I. Varieties.				
T. vulgare:				
0984 d.....	beardless	white, glabrous	red	var. *lutescens* KÖRN.
Sol III	»	» »	»	»
Ankar I	»	» »	»	»
Extra-Squarehead II	»	» »	»	»
Carsten V.....	»	» »	»	»
Minhardi	»	» »	»	»
Kron	»	» »	»	»
Äring I	»	» »	»	»
Drott	»	» »	»	»
Pansar	»	» »	»	»
Skandia I	»	» »	»	»
Standard	»	» »	»	»
Sv 23/54.....	»	» »	»	»
Wilhelmina ...	»	» »	white	var. *albidum* KÖRN.
Sammet (0700)	»	» pubescent	red	var. *velutinum* KÖRN.
Gluten	»	» »	»	»
Pudel	»	» »	white	var. *leucospermum* KÖRN.
0761	»	red, glabrous	red	var. *milturum* KÖRN.
01201	bearded	white, »	»	var. *erythrospermum* KÖRN.
Bearded Pansar	»	» »	»	Pansar mutation
T. compactum:				
Glabrous Club	beardless	» »	»	var. *Wernerianum* KÖRN.
Pubescent Club	»	» pubescent	»	var. *Willmackianum* KÖRN.
Blue Club.....	»	blue-black, pubescent	»	var. *atrum* KÖRN.
Bearded Club	bearded	white, glabrous	»	var. *icterinum* KÖRN.
T. Spelta:				
Hohenheimer Spelz	beardless	» »	»	var. *album* KÖRN.
BeardlessSpelt	»	red »	»	var. *Duhamelianum* KÖRN.
Blue Spelt.....	»	grey-blue, pubescent	»	var. *Alefeldii* KÖRN.
White Spelt ...	bearded	white, glabrous	»	var. *Arduini* KÖRN.
Bearded Spelt	»	red »	»	var. *vulpinum* KÖRN.
T. turgidum:				
Lusitanicum...	bearded, simple	white, glabrous	white	var. *lusitanicum* KÖRN.

	Ear	Glumes	Grain	
Speciosum ...	bearded, simple	red, glabrous	red	var. *speciosum* KÖRN.
Dinurum	bearded, simple	» pubescent	»	var. *dinurum* KÖRN.
Red Rivet.....	bearded, simple	» »	»	»
Rivet Cambr. 4	bearded, simple	blue-black, pubescent	»	var. *iodurum* KÖRN.
Trigo Cajudo	bearded, branched	red, glabrous	white	var. *pseudocervinum* KÖRN.
Compositum...	bearded, branched	» pubescent	red	var. *Linnæanum* KÖRN.
II. Lines.				
Vulgare-like:				
6009/11	bearded	white, glabrous	»	
6051/64	beardless	» pubescent	»	
6068	»	» »	»	
6103/104	»	red »	»	
6229/231	»	white »	»	
6240/244	»	» glabrous	»	
Spelta-like:				
Beardless Spel-toid	beardless	» »	»	Pansar mutation
Bearded Spel-toid	bearded	» »	»	» »
6017	beardless	» »	»	
6019/21	bearded	heteroz., pubescent	»	
6019	»	red, pubescent	»	
6020	»	» »	»	
6021	»	» »	»	
6024/26	»	heteroz. »	»	
6024	»	» »	»	
6163/167	»	red, glabrous	»	
6163	»	» »	»	
6174	beardless	white »	»	
6177	»	» »	»	
6178	»	» »	»	
6179/185	heteroz.	red »	»	
Turgidum-like:				
6043	beardless	» pubescent	»	*contractum*
6153/157	heteroz.	white, heteroz.	»	*contractum*

	Ear	Glumes	Grain	
6153	heteroz.	white, pubescent	red	*contractum*
6157	»	white, pubescent	»	*contractum*
6214/217	bearded	red, glabrous	»	
6221	»	white »	»	*contractum*
6223/225	heteroz.	heteroz., pubescent	»	

tion of the origin and properties of these last-mentioned lines will be given later on (pp. 305—309).

Among the parental varieties of the table are also three homozygous mutants from Prof. NILSSON-EHLE's material, namely a bearded normal, a bearded and a beardless speltoid, all out of Pansar.

Fig. 1. The parental varieties of cross No. *1*. To the left, 0984 d *vulgare*; to the right, Red Rivet *turgidum*.

The parental varieties have been crossed in different combinations, as will be seen in Table 2. Most of these crosses are only dealt with briefly in this paper as to fertility or segregation of some morphological characters. Some of the crosses, however, have been used as starting-material for the physiological investigations, as will be seen from the column furthest to the right, where the numbers of the lines extracted from these crosses are given. The crosses belong to several different species combinations. Thus, *vulgare* has been crossed with *turgidum* 14 times in 11 different varietal combinations, the cross 0984 d × Red Rivet being repeated 4 times. In

TABLE 2. *Varietal combinations in the crosses.*

Number of the cross	Species combin.	Varietal combinations ♀ × ♂	Year of crossing	Lines derived from the cross
1	V × T	0984d × Red Rivet	1931	6003—6244
5	»	Sol III × Compositum *a. rec.*	1934	8003—8031, 8104—8127
6	»	Sol III × Red Rivet *a. rec.*	»	8035—8042
7	»	Ankar I × Red Rivet *a. rec.*	»	8046—8057
8	»	Pudel × Red Rivet *a. rec.*	»	8061—8064
9	»	Gluten × Red Rivet *a. rec.*	»	8068—8077
10	»	0761 × Red Rivet *a. rec.*	»	8081—8087
11	»	0984d × Red Rivet *a. rec.*	»	8091—8099
32	»	Skandia I × Rivet Cambr. 4 *a. rec.*	1935	
66	»	Wilhelmina × Dinurum *a. rec.*	1937	0002—0028
67	»	Minhardi × Rivet Cambr. 4 *a. rec.*	»	0035—0063
69	»	0984d × Red Rivet *a. rec.*	»	
77	»	0984d × Red Rivet	1938	
82	»	Sv 23/54 × Compositum	1927	
16	(V × T) × V	(Sol III × Compos.) F_1 × Sol III	1935	9002—9012
18	»	(Sol III × Red Riv.) F_1 × Sol III	»	9025—9029
19	»	(Gluten × Red Riv.) F_1 × Gluten	»	9031—9032
20	»	(Pudel × Red Riv.) F_1 × Pudel	»	9034—9036
22	»	(0984d × Red Riv.) F_1 × 0984d	»	9049—9059
41	(V × T) × S	(Sol III × Red Riv.) F_1 × White Spelt	»	
42	(V × T) × C	(Sol III × Red Riv.) F_1 × Bearded Club	»	
17	(V × T) × T	(Sol III × Compos.) F_1 × Compos.	»	9014—9023
21	»	(Pudel × Red Riv.) F_1 × Red Rivet	»	9038—9047
23	»	(0984d × Red Riv.) F_1 × Red Rivet	»	9061—9067
40	V × (V × T)	0984d × (0984d × Red Riv.) F_1	»	
39	T × (V × T)	Red Rivet × (0984d × Red Riv.) F_1	»	
24	V × VVT	01201 × 6009/11 *a. rec.*	»	9070—9075
25	»	Carsten V × 6240/244 *a. rec.*	»	9078—9082
26	»	Sammet × 6051/64 *a. rec.*	»	9085—9093
27	»	Extra-Squarehead II × 6103/104	»	9096—9103
62	»	Sammet × 6068 *a. rec.*	1936	
28	T × TVT	Red Rivet × 6214/217 *a. rec.*	1935	9104—9114
33	»	Speciosum × 6223/225 *a. rec.*	»	
57	T × TcVT	Lusitanicum × 6043	1936	
30	S × SVT	White Spelt × 6163/167	1935	9126—9131
31	»	Blue Spelt × 6179/185 *a. rec.*	»	9135—9139
43	»	6017 × White Spelt	1936	
45	»	White Spelt × 6019 *a. rec.*	»	
48	»	6024 × Blue Spelt	»	
50	»	Hohenheimer × 6163 *a. rec.*	»	

Number of the cross	Species combin.	Varietal combinations ♀ × ♂	Year of crossing	Lines derived from the cross
52	S × SVT	6174 × Hohenheimer	1936	
53	»	6177 × White Spelt	»	
34	T × VVT	Red Rivet × 6229/231 *a. rec.*	1935	
35	T × SVT	Rivet Cambr. 4 × 6019/21 *a. rec.*	»	
36	»	Rivet Cambr. 4 × 6024/26 *a. rec.*	»	
44	»	6017 × Dinurum	1936	
46	»	6020 × Rivet Cambr. 4	»	
47	»	Rivet Cambr. 4 × 6021	»	
29	C × TcVT	Bearded Club × 6221	1935	9115—9123
37	»	6153/157 × Pubescent Club	»	
58	»	6157 × Glabrous Club	1936	
59	»	Glabrous Club × 6153	»	
15	V × V	Pansar × Bearded Pansar *a. rec.*	1934	
83	V × C	Standard × Blue Club	1927	
2	V × S	Kron × Bearded Spelt	1931	
3	»	Kron × Beardless Spelt	»	
4	»	Standard × Bearded Spelt	»	
12	»	Äring I × Blue Spelt *a. rec.*	1934	
13	»	Drott × Hohenheimer *a. rec.*	»	
80	S × T	Hohenheimer × Compositum *a. rec.*	1938	
60	T × T	Compositum × Trigo Cajudo *a. rec.*	1936	
70	»	Dinurum × Red Rivet *a. rec.*	1937	
68	S × SV	White Spelt × Beardless Speltoid *a. rec.*	»	0067—0076
63	SV × SVT	6177 × Beardless Speltoid	1936	
64	»	Beardless Speltoid × 6178 *a. rec.*	»	
75	»	Bearded Speltoid × 6178 *a. rec.*	1937	
76	»	6178 × Beardless Speltoid	»	

Abbreviations: V = *vulgare*; T = *turgidum*; C = *compactum*; S = *Spelta*; VVT = *vulgare*-like, TVT = *turgidum*-like, TcVT = *turgidum-compactum*-like (»*contractum*«), SVT = *Spelta*-like (»*speltoides*«) line out of V × T cross No. *1*; SV = speltoid mutant out of V; *a. rec.* = and reciprocally.

11 different combinations F_1 of *vulgare* × *turgidum* has been used for back-crosses, and in no less than 30 cases 6000-lines have been crossed with varieties of different species. Finally, 11 other inter- and intraspecific crosses are included in the table. Abbreviations used in this table and in the text are explained at the foot of the table.

The material has been used for investigations on the following points:

(1) The seed production and vitality in different crosses.

(2) The segregation of some morphological characters with special regard to the *speltoides* complex.

(3) The chromosome numbers in different types of the offspring.

(4) The cold-resistance in hexaploid and tetraploid progenies.

(5) Some other physiological characters and their correlation to the chromosome number.

In the treatment of the material statistical methods have been employed on several occasions. The significance of found values has been tested according to BONNIER and TEDIN (1940). At the estimation of the significance of differences and in other cases when the table of t has been used, the values of t have been given with asterisks or noughts as exponents in the following way, $t^{***} = P < 0{,}001$, $t^{**} = P\ 0{,}01$ —$0{,}001$, $t^{*} = P\ 0{,}05$—$0{,}01$, $t^{0} = P\ 0{,}1$—$0{,}05$, $t^{00} = P\ 0{,}2$—$0{,}1$, $t^{000} = P > 0{,}2$. The same signs have also been used in the χ^2 tests to mark the P-values.

Before giving an account of the different experiments and their results it seems necessary to give a short survey of the very comprehensive literature on the cytogenetics of pentaploid wheat hybrids.

3. PENTAPLOID WHEAT CROSSES AND THEIR CYTOGENETICS.

The discovery of polyploidy in wheat made it possible to explain many riddles which had presented themselves in certain species crosses. Thanks to KIHARA, WATKINS, MATSUMURA, SAX and others the cytogenetics is now unravelled as a principle.

The cross between hexaploid and tetraploid *Triticum* usually succeeds quite easy and results in a pentaploid F_1, the chromosomes of which group themselves in 14 bivalents and 7 univalents at meiosis. Since the tetraploid wheat species are presumed to possess the genomes *A* and *B* (*T. Timopheevi A* and *G*, according to LILIENFELD and KIHARA, 1934), and the hexaploid species *A*, *B*, and *C* (or *ABD* in another terminology), the bivalents are supposed to come from the homologous genomes *A* and *B*, the univalents, on the other hand, from the overlapping or »extra» genome *C*. After self-fertilization the F_2 ought to contain all numbers from 28 to 42 in frequencies according to the binomial distribution, if no disturbances occur. According to MATSUMURA (1936 a, 1939), who has investigated the somatic chromosome numbers of about 900 F_2 plants out of *Spelta* × *polonicum*, *vulgare* × *durum*, and *compactum* × *persicum*, all numbers from 28 to 42 are certainly represented, but the frequency curve deviates from the ideal distribution by a certain flattening of the top and some other irregularities, which will be further discussed later on (see p. 292 and

Fig. 3). The deviations seem to depend especially upon the elimination of univalents, different fertilization ability among the pollen-grains, and failing germination of several embryos with intermediate chromosome numbers (KIHARA and MATSUMURA, 1940). According to their chromosome number, KIHARA (1924) has divided the F_2 plants into two groups, the increasing group (»Vermehrungsgruppe«) with more than 35, and the decreasing group (»Verminderungsgruppe«) with less than 35 chromosomes. In the former group zygotes with 21 units at meiosis — that is the sum of number of bivalents and number of univalents — are more or less fertile and vigorous and are called »fertile combinations« in contrast to all other possible chromosome constellations, which are »sterile combinations« and usually die out in different stages of development. In the decreasing group zygotes with 14_{II} and $0—7_{I}$ represent fertile combinations, whereas other combinations are sterile. The result of this is that plants belonging to the increasing group give a progeny with the same number or higher until 42 is reached, while plants belonging to the increasing group finally, after a number of generations, have a 28 chromosome progeny. Constant lines with other numbers than 42 and 28 are very rare, but a few cases are described (cf. MATSUMURA, 1939).

Even if the final result of a pentaploid wheat cross thus means a reversion to the chromosome numbers of the parents, the existence of homologous genomes will make itself known in the shape of clear recombinations of a great number of characters. Several morphological qualities, e. g. the colour, the pubescence and the beardness of the heads, the grain colour, are inherited only or practically only by genes located in the *A*- and *B*-genomes, and in F_2 they give Mendelian segregation ratios. Other characters, however, e. g. the waxiness of the leaves, the keel of the glumes, the resistance to yellow rust (WATKINS, 1930), and the solidity of straw (YAMASHITA, 1937) are also regulated by the *C*-genome.

From the plant breeders' point of view the pentaploid wheat crosses have been the subject of great interest and extensive investigations. It is a well-known fact that varieties of the tetraploid species *durum*, *dicoccum*, and *persicum* possess a better resistance to fungous diseases than *vulgare*. Many attempts have also been made to transfer this quality to the common *vulgare* wheats by means of crossing. As examples of successful breeding of this kind the varieties Marquillo (out of *vulgare* Marquis × *durum* Iumillo; HAYES *et al.*, 1920) and Hope (out of *vulgare* Marquis × *dicoccum* Yaroslav; MCFADDEN, 1930) will

be mentioned. Both these varieties were marketed in the nineteen-twenties in America.

4. SEED DEVELOPMENT IN RECIPROCAL CROSSES OF T. VULGARE × TURGIDUM AND OTHER COMBINATIONS.

In the literature relating to interspecific crosses in *Triticum* the seed setting and development after artificial pollination are described by several authors. The most important contributions have been made by WATKINS (1927 a, 1932), THOMPSON *et al.* (THOMPSON and CAMERON, 1928; THOMPSON, 1930; BOYES and THOMPSON, 1937), and WAKAKUWA (1930, 1934). WATKINS found that the cross hexaploid ♀ × tetraploid ♂ gave grains with a germination of 84 %, whilst the grains from the reciprocal cross only germinated in 38 % on an average. The frequency of grain formation in the pollinated heads is not recorded. In back-crosses of F_1 of *vulgare* × *turgidum* with the parents reciprocally he found that F_1 ♀ × *vulgare* ♂ always gave shrivelled and poorly germinating grains and that *turgidum* ♀ × F_1 ♂ also gave many shrivelled grains. On the other hand, *vulgare* ♀ × F_1 ♂ and F_1 ♀ × *turgidum* ♂ gave better seed development. The best germination was thus obtained when the higher chromosome number belonged to the mother plant. According to WATKINS, the development of the grains depends upon the balance between the embryo and the endosperm and the chromosome numbers of these tissues. In the cross *vulgare* × *vulgare* the embryo has 6x chromosomes and the endosperm on account of the fusion of two polar nuclei and a male gamete 9x. In the cross *turgidum* × *turgidum* the corresponding quantities are 4x and 6x, and the quotient is thus 2 : 3 in crosses between varieties with equal chromosome numbers. When *vulgare* and *turgidum* are crossed, however, the embryo and the endosperm will contain respectively 5x and 8x if the hexaploid parent is used as female, and 5x and 7x if the tetraploid is female. In the former case the 8x endosperm is developed on a 6x plant (which normally at self-fertilization carries 9x endosperms) and »though too small, is properly nourished and well formed». In the latter case the 7x endosperm, »which has too many chromosomes» (growing on a 4x plant), »is poorly nourished and develops imperfectly». In spite of WATKINS's formulation, cited above, he concludes that he cannot agree with MÜNTZING's (1930) supposition, which is based upon the conditions in *Galeopsis* and a number of other examples, that the seed development in crosses between parents with different chromosome numbers is

dependent upon the chromosomal quotients of all the three tissues, embryo, endosperm, *and* mother plant.

THOMPSON and CAMERON (1928) found that in the cross *vulgare* ♀ × *durum* ♂ 84,2 % of the grains obtained germinated but in the reciprocal cross only 55,9 %. No comparable figures of the original seed setting are given. They also studied some other similar species crosses and different back-crosses. The result of the back-crosses agreed with WATKINS's, F_1 ♀ × hexaploid ♂ and tetraploid ♀ × F_1 ♂ giving poor germination in contrast to hexaploid ♀ × F_1 ♂ and F_1 ♀ × tetraploid ♂, which germinated fairly well. In a later paper THOMPSON (1930) declares that the best original seed setting was obtained in the back-crosses when F_1 was used as mother plant. In back-crosses of F_1 with *vulgare* plumper seeds with better germination were usually obtained when *vulgare* was female than in the reciprocal cross, but »in back-crosses with emmers also the seeds are better when the pure parent is female». The last-mentioned result is in conflict with the conclusions of THOMPSON and CAMERON (1928).

MEISTER (1928) studying *vulgare* × *durum* concludes: »Es konnte bestätigt werden, dass bei der Kreuzung mit *Tr. durum* als Mutterpflanze die Kreuzungsfähigkeit höher ist, bei der reziproken Kreuzung jedoch die Keimfähigkeit der Samen».

In WAKAKUWA's (1930, 1934) investigations on different pentaploid crosses hexaploid ♀ × tetraploid ♂ set 71,07 % of grains, 98,18 % of which germinated. On the other hand, tetraploid ♀ × hexaploid ♂ showed a better seed setting with 95,28 % but a less good germination with only 61,26 %.

Similar results have also been obtained by KATAYAMA (1933), who crossed *vulgare* ♀ × *pyramidale* ♂ (91,67 % seed setting, 90,91 % germination) and *pyramidale* ♀ × *vulgare* ♂ (96,43 % seed setting, only 7,41 % germination). He also tried to cross *vulgare* and *Timopheevi*; in the direction 4x ♀ × 6x ♂ he got full seed setting (20 grains in 20 flowers), but the seeds did not germinate, in the other direction he got no seeds at the pollination. Remembering the special genome constitution of *Timopheevi*, these results are not so astonishing.

MATSUMURA (1939) also reports better seed setting but less good germination in *durum* ♀ × *vulgare* ♂ than in the reverse cross. His material was, however, very small. In back-crosses between F_1 of *vulgare* × *durum* and the parents MATSUMURA (1939) found that the seed setting was highest in the combinations F_1 ♀ × *vulgare* ♂ and

durum ♀ × F_1 ♂, but that the germination was better in the reverse crosses with high-chromosomal mother plants.

Summarizing the results of the previous authors, we find that the best seed setting was obtained when the mother plant had the lower chromosome number, but that the grains obtained from the higher chromosomal mother plants had a better development and germination.

In my own material the seed setting has also been calculated from the number of obtained grains and the number of pollinated flowers. The germination in the field was not always controlled, but a measure of the viability of the grains sown has been obtained by counting the number of plants surviving at the time of the harvest. No disturbing cold damages occurred. The different figures are collected in Table 3.

In order to make it possible to compare seed setting and grain viability in reciprocal crosses averages are calculated in some cross groups. These averages, however, only refer to those crosses in which identical varieties are used in both combinations. Thus the percentage of harvested plants is only compared where grains of both the reciprocal crosses are really sown.

In the combination *vulgare* × *turgidum* there are 11 comparisons of seed setting in reciprocal crosses. As shown in Table 3, *vulgare* ♀ × *turgidum* ♂ has been superior in nine cases and on an average the cross succeeds about three times better in this direction than in the other one (16,4 % and 5,2 % respectively). The difference in seed setting percentage is rather significant, as the inter-class and intra-class variation at an analysis of variance show a quotient of $5{,}90^{*}$ (D. F. 1 and 20 respectively).

Also in the germination ability and the vitality during the vegetation period the zygotes derived from *vulgare* ♀ × *turgidum* ♂ are better than the zygotes from the reciprocal cross. This is quite clear in all the seven crosses, and the averages are so different as 72,4 and 18,5 % harvested plants. The quotient for inter- and intra-class variance is here $47{,}95^{***}$ (D. F. 1 and 12).

These figures of seed setting and vitality can be compared with the results of crossing within *vulgare*. In my own material only one cross of this type (No. *15*, normal Pansar × bearded mutation out of Pansar) has been studied, where the seed setting was 26,3 % in one direction and 34,4 % in the other one, while the percentage of harvested plants obtained was 76,1 and 76,3 % respectively. In the practical breeding work at the Wheat and Oat Department of the Swedish Seed Association a great number of crosses within *vulgare* have been carried out at Svalöf during the same years, 1934—1938 (Table 4).

TABLE 3. *The seed setting and vitality in reciprocal crosses.*

Number of the cross	Year	Number of pollinated flowers		Seed setting percentage		Harvested F_1 plants in % of grains sown	
		V × T	T × V	V × T	T × V	V × T	T × V
5	1934	135	243	7,4	2,9	80,0	9,1
6	»	108	135	10,2	1,5	69,2	0
7	»	162	135	3,1	20,0	80,0	48,1
8	»	135	108	14,1	6,5	78,9	28,6
9	»	216	135	19,0	4,4	75,6	28,6
10	»	135	135	8,9	0	78,6	—
11	»	135	135	11,1	20,0	73,3	15,0
32	1935	56	144	7,1	2,1	50,0	0
66	1937	32	130	25,0	0	76,9	—
67	»	130	64	50,0	0	80,6	—
69	»	124	166	24,2	0	54,8	—
77	1938	304	—	5,3	—	75,0	—
Average of reciprocals [1]				16,4	5,2	72,4	18,5
		V × F_1	F_1 × V	V × F_1	F_1 × V	V × F_1	F_1 × V
16	1935	—	228	—	15,4	—	40,9
18	»	—	82	—	8,5	—	42,9
19	»	—	143	—	9,1	—	7,7
20	»	—	102	—	2,9	—	33,3
22, 40	»	274	160	2,2	26,3	25,0	11,9
		T × F_1	F_1 × T	T × F_1	F_1 × T	T × F_1	F_1 × T
17	1935	—	254	—	9,1	—	38,1
21	»	—	154	—	26,6	—	60,8
23, 39	»	284	120	1,8	14,2	0	29,4
		V × VVT	VVT × V	V × VVT	VVT × V	V × VVT	VVT × V
24	1935	112	106	0,9	13,2	0	57,1
25	»	66	86	25,8	0	52,0	—
26	»	129	149	0	2,7	—	25,0
62	1936	122	118	35,2	36,4	79,1	81,4
Average of reciprocals [1]				15,5	13,1	—	—
		T × TVT	TVT × T	T × TVT	TVT × T	T × TVT	TVT × T
28	1935	70	160	18,6	16,9	38,5	25,9
33	»	123	164	0	1,2	—	0
57	1936	130	—	30,8	—	32,5	—
Average of reciprocals [1]				9,3	9,1	—	—
		S × SVT	SVT × S	S × SVT	SVT × S	S × SVT	SVT × S
30	1935	84	—	2,4	—	100,0	—
31	»	78	134	0	0,7	—	100,0

[1] In the average are only those crosses included where reciprocal comparison is possible. See text.

Number of the cross	Year	Number of pollinated flowers		Seed setting percentage		Harvested F_1 plants in % of grains sown	
43	1936	—	120	—	36,7	—	95,5
45	»	116	96	41,2	16,7	66,7	84,2
48	»	—	104	—	22,1	—	75,0
50	»	112	108	15,2	13,0	58,8	57,1
52	»	—	94	—	12,8	—	50,0
53	»	—	95	—	8,4	—	55,6
Average of reciprocals [1]				18,8	10,1	62,8	70,7
		T × VVT	VVT × T	T × VVT	VVT × T	T × VVT	VVT × T
34	1935	138	136	0,7	22,1	0	30,0
		T × SVT	SVT × T	T × SVT	SVT × T	T × SVT	SVT × T
35	1935	96	80	0	8,8	—	14,3
36	»	76	66	0	56,1	—	32,5
44	1936	—	72	—	16,7	—	71,4
46	»	—	124	—	23,4	—	48,3
47	»	130	—	6,2	—	0	—
		C × TcVT	TcVT × C	C × TcVT	TcVT × C	C × TcVT	TcVT × C
29	1935	56	—	12,5	—	42,9	—
37	»	—	94	—	1,1	—	0
58	1936	—	132	—	2,3	—	0
59	»	76	—	15,8	—	75,0	—
		V′ × V″	V″ × V′	V′ × V″	V″ × V′	V′ × V″	V″ × V′
15	1934	270	270	26,3	34,4	76,1	76,3
		V × S	S × V	V × S	S × V	V × S	S × V
12	1934	243	81	40,3	51,9	80,6	73,8
13	»	270	189	4,8	10,6	64,3	80,0
Average of reciprocals [1]				22,6	31,3	72,5	76,9
		S × T	T × S	S × T	T × S	S × T	T × S
80	1938	194	81	3,1	8,6	50,0	0
		T′ × T″	T″ × T′	T′ × T″	T″ × T′	T′ × T″	T″ × T′
60	1936	125	114	21,6	8,8	0	0
70	1937	90	64	15,6	6,3	83,3	66,7
		S × SV	SV × S	S × SV	SV × S	S × SV	SV × S
68	1937	52	74	17,3	6,8	87,5	50,0
		SV × SVT	SVT × SV	SV × SVT	SVT × SV	SV × SVT	SVT × SV
63	1936	—	25	—	16,0	—	75,0
64	»	24	23	0	43,5	—	70,0
75	1937	30	58	0	24,1	—	83,3
76	»	—	60	—	8,3	—	50,0

[1] In the average are only those crosses included where reciprocal comparison is possible. See text.

TABLE 4. *Seed setting in crosses within vulgare at Svalöf.*

Year	Number of cross comb.	Number of pollinated flowers	Seed setting percentage max.	min.	average
1934	47	13385	76,4	1,2	39,7
1935	62	10526	85,9	0,7	40,2
1936	60	8489	76,9	2,9	43,5
1937	35	8879	79,9	15,3	44,0
1938	42	5592	74,8	3,1	32,6
Total	246	46871	—	—	—
Average	—	—	—	—	40,0

The average seed setting at these crosses, which have been carried out in the same years and partly by the same persons as in my own crosses, is 40,0 %. There are, however, very great fluctuations between different varietal combinations. A clear tendency is that the earliest varietal combinations, which are usually crossed about June 20th, have a very low seed setting. The late combinations, on the other hand, which were carried out about July 1st, show a higher seed setting. On an average the early crosses have given about 20 % and the late ones about 50 % seed setting in this breeding material.

The crosses between *vulgare* and *turgidum* were carried out about July 1st, and hence their seed setting of 16,4 % (V × T) and 5,2 % (T × V) should be compared with the 50 % in the late *vulgare* crosses. In *vulgare* crosses the percentage of harvested plants in relation to the number of grains sown is usually about 70—90 %. In the crosses *vulgare* ♀ × *turgidum* ♂ the viability of the grains is consequently fairly good, while the viability of the grains set in the reciprocal crosses must be considered as very low.

The seed setting in the back-crosses was poor in the combinations *vulgare* ♀ × F_1 ♂ and *turgidum* ♀ × F_1 ♂, but considerably better in the reciprocal crosses. In comparable crosses the vitality of the grains was lower in *turgidum* ♀ × F_1 ♂ (0 %) than in F_1 ♀ × *turgidum* ♂ (29,4 %), but better in *vulgare* ♀ × F_1 ♂ (25,0 %) than in F_1 ♀ × *vulgare* ♂ (11,9 %). As to the last-mentioned cross there are, however, four other combinations (crosses Nos. *16*, *18*, *19*, *20*) without reciprocals, among which the number of harvested plants was relatively high in three cases.

In the crosses with equal parental chromosome numbers (hexaploid: V × V, S × V, V × VVT, S × SVT, SV × SVT; tetraploid: T × T, T × TVT) both the seed setting and the vitality have been about

the same in both directions in all cases except one. The combination SV ♀ × SVT ♂, though repeated two years, gave no grains, but in the opposite direction seeds were set. An explanation of this exception from the general rule, which has been advanced by previous authors, cannot be given here. It must be added, however, that the number of pollinated flowers was low in the crosses Nos. *64* and *75*, where SVT 6178 was crossed with two different homozygous speltoids.

There are also a number of other crosses, all pentaploid, included in Table 3, viz. VVT × T, SVT × T, C × TcVT, and S × T. In all these crosses except S × T the seed setting and the vitality have both been very poor when the tetraploid parent was used as mother plant, and considerably better in the reverse crosses, though direct comparison is not always possible. In the cross S × T more grains were set in tetraploid ♀ × hexaploid ♂, but none of them gave fully developed plants, whilst the seeds of the reverse cross showed good viability.

The results obtained in my own material differ in one respect remarkably from those of the previous authors. As already mentioned, these investigators found that the seed setting was higher in the direction low ♀ × high chromosome parent ♂, but that the germination of the grains was better in the reverse direction. KATAYAMA (1933) has compared seed setting and germination in crosses of species with different chromosome numbers. For the setting of seeds he gives a scheme of three different types, namely »the *Datura* type» (high numbered ♀ better than low numbered ♀), »the *Triticum* type» (low numbered ♀ better than high numbered ♀), and »the *Brassica* type», where the two former types are seen in the same genus. As to the germination he states that ordinarily the result is better in high numbered ♀ than in low numbered ♀, but that in some genera the conditions are the reverse (e. g. *Nicotiana*) or no differences occur (e. g. *Brassica*). It is interesting to note that according to KIHARA and NISHIYAMA (1932) *Avena* follows the »*Triticum* type».

As shown in Table 5, the deviation from the »*Triticum* type» of KATAYAMA is clearly documented in my material, which must be considered as sufficiently comprehensive. In this concluding table values from all the pentaploid crosses from different years have been thrown together, which seemed legitimate in this connection since no visible differences between the results of the years in question appear.

The reasons for the deviation can be of different kinds. Firstly, it is possible that *vulgare* × *turgidum* behaves in another way than the species combinations studied by WAKAKUWA and KATAYAMA.

TABLE 5. *Comprehension of the seed development in some pentaploid crosses.*

	Number of flowers pollinated		Number of grains obtained		Number of plants harvested	
	6x ♀	4x ♀	6x ♀	4x ♀	6x ♀	4x ♀
vulgare × *turgidum* ..	1548	1530	220	79	162	22
VVT × *turgidum*	136	138	30	1	0	0
SVT × *turgidum*	342	302	85	8	36	0
compactum × TcVT ..	132	226	19	4	12	0
Spelta × *turgidum* ...	194	81	6	7	3	0
Total	2352	2277	360	99	213	22
Grains/flowers %	—	—	15,3	4,3	—	—
Plants/grains %	—	—	—	—	59,2	22,2

Secondly, my own cross pollinations have been carried out under open field conditions, where the seed setting has been depressed by the weather and the scantiness of pollen. This explains the low absolute figures for the seed setting, but there is also some reason to presume that the crosses 4x ♀ × 6x ♂ have been specially disfavoured by these circumstances. It is namely well known from the breeding experiments at Svalöf that the tetraploid wheat species do not thrive very well in our climate, which means that the tetraploid mother plants may be handicapped to a certain degree in comparison with the hexaploid mother plants. As seen in Table 3, the crosses T × T show a relatively low seed production, which may depend upon special frailties caused by castration and isolation.

Since no special investigations have been devoted to the problem of the ultimate reasons of the different reciprocal production of viable seeds, I am not able to discuss these things closer. However, since the embryo is nourished by the endosperm and the endosperm by the mother plant, it seems to me that chromosomal balance between all of these three tissues must be decisive for the development of the zygote (cf. MÜNTZING, 1930, 1933).

In the back-crosses my own results are based on a very limited material. The development of the seeds seemed to vary in a very high degree, which must be ascribed to the pronounced chromosomal unbalance in many combinations. The figures obtained in the reciprocals of *vulgare* × F_1 are, however, in agreement with MATSUMURA's findings of better seed setting on the low-chromosomal mother plant but worse germination of the grains sown. In *turgidum* × F_1 the seed setting in

turgidum ♀ has failed, which disagrees with the rule, but may be due to frailty caused by the crossing manipulations.

5. MORPHOLOGY AND FERTILITY IN F_1 PLANTS.

In pentaploid wheat crosses F_1 is intermediate in appearance, except in such quantitative characters as are influenced by the typical hybrid vigour that appears. This is well-known from the investigations of previous authors but has also become manifest in my own material.

In Table 6 some figures concerning plant height, number of culms per plant, and number of grains per head are given for the parental varieties and for F_1 in 1935.

As seen in Table 6, the F_1 plants are on an average just as high or a little higher than the highest parent, *turgidum*. Respecting the number of culms per plant it is well-known that *turgidum* has a relatively low tillering capacity. F_1 is also in this respect luxuriant. The fertility of the material given in Table 6 is not investigated in detail, only the number of grains per head being recorded. In general, *turgidum* has very long heads with many spikelets and also with a high number of grains per head. The *vulgare* varieties used in these crosses have shorter heads and

TABLE 6. *Vigour and fertility in F_1 and its parents.*

Vulgare				*Turgidum*				F_1			
Variety	Number of plants	Height cm.	Culms per plant	Grains per head	Variety	Number of plants	Height cm.	Culms per plant	Grains per head	Cross No.	Number of plants	Height cm.	Culms per plant	Grains per head
Sol III..	29	121	9,6	32,2	Compositum	29	138	9,5	53,4	5	9	150	13,3	24,4
» » ..	27	115	7,5	32,7	Red Rivet..	21	139	4,6	48,4	6	12	140	11,3	19,6
Ankar I	29	123	10,8	34,2	» ..	15	141	5,8	48,7	7	18	139	10,4	26,2
Pudel ..	31	132	10,5	30,3	» ..	19	150	8,9	43,3	8	15	154	8,9	26,2
Gluten..	29	109	8,6	27,3	» ..	25	138	5,6	51,9	9	33	135	10,0	27,4
0761 ...	19	123	13,4	20,4	» ..	19	138	6,1	48,2	10	11	144	10,2	29,4
0984 d..	33	117	9,8	33,1	» ..	37	141	7,5	49,6	11	17	137	9,4	18,2
Total	197	—	—	—		165	—	—	—		115	—	—	—
Average	—	120,0	10,0	30,0		—	140,7	6,9	49,1		—	142,7	10,5	24,5

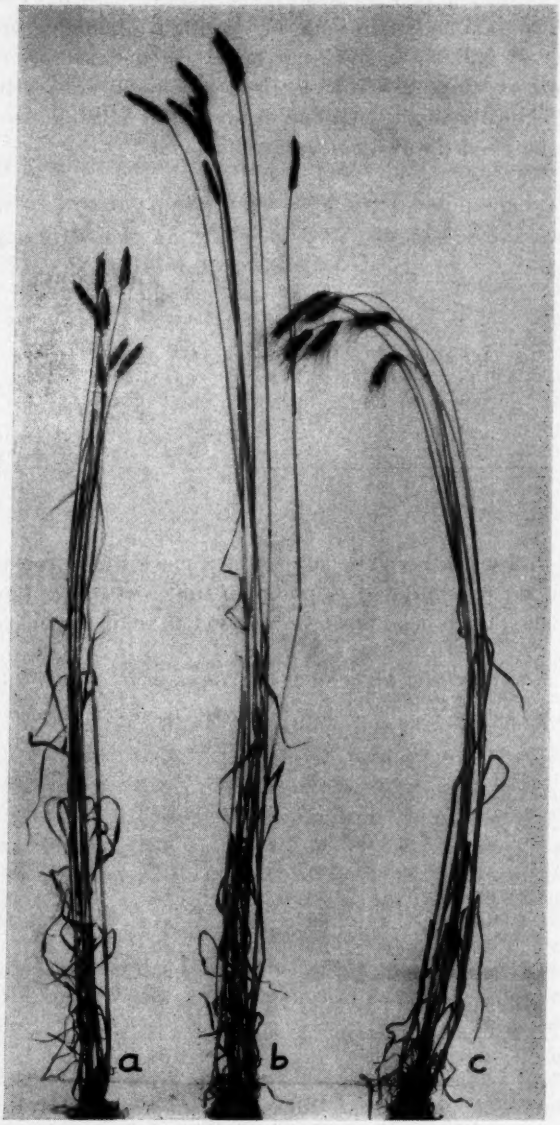

Fig. 2. Hybrid vigour in F_1 of cross No. 8. a = Pudel *vulgare*, b = F_1, c = Red Rivet *turgidum*.

fewer spikelets and grains per head. The hybrid plant has even longer heads than *turgidum*, but in spite of the high number of spikelets a low number of grains are given per head. In the case of five of the combinations of Table 6 F_1 plants from reciprocal crosses have been obtained. The comparison made in Table 7 does not point to any real differences between the two categories of plants.

TABLE 7. *Vigour and fertility in F_1 plants from reciprocal crosses.*

	Vulgare ♀ × *turgidum* ♂				*Turgidum* ♀ × *vulgare* ♂			
Cross No.	Number of plants	Height cm.	Culms per plant	Grains per head	Number of plants	Height cm.	Culms per plant	Grains per head
5	8	151	13,1	25,6	1	145	15,0	16,1
7	4	133	12,5	24,6	14	140	9,8	26,8
8	13	156	8,9	25,2	2	140	9,0	32,9
9	31	136	10,0	27,6	2	128	9,0	23,8
11	11	139	7,2	18,8	6	135	13,5	17,6
Total	67	—	—	—	25	—	—	—
Average	—	143,0	10,3	24,4	—	137,6	11,3	23,4

The fertility has been the subject of a close study in a number of heads from the parental and F_1 plants of the cross No. *8*, Pudel × Red Rivet, in 1935. Each head has been selected as being the biggest normal

TABLE 8. *Seed setting percentage per head in F_1 plants and parents.*

	Pudel, *vulgare*			Red Rivet, *turgidum*			F_1, Pudel × Red Rivet		
Head No.	Number of flowers	Number of grains	Seed setting %	Number of flowers	Number of grains	Seed setting %	Number of flowers	Number of grains	Seed setting %
1	48	38	79,2	77	67	87,0	81	38	46,9
2	77	51	66,2	73	61	83,6	69	40	58,0
3	74	51	68,9	78	69	88,5	76	38	50,0
4	59	40	67,8	80	65	81,3	79	44	55,7
5	63	51	81,0	61	49	80,3	80	36	45,0
6	37	31	83,8	76	61	80,3	70	32	45,7
7	70	51	72,9	80	61	76,3	97	49	50,5
8	55	42	76,4	—	—	—	75	38	50,7
Average	60,4	44,4	74,5	75,0	61,9	82,5	78,4	39,4	50,3

head of the plant. As seen in Table 8, the *vulgare* parent, Pudel, had a seed setting of 74,5 %, while Red Rivet *turgidum* had a little more, or 82,5 %. The F_1 heads on an average set only 50.3 %. The average

number of flowers per head was highest in the F_1 plants. The variation between the different heads is very low within each plant type.

For comparison some fertility figures calculated by MATSUMURA (1936 a) for the F_1 of the cross *Spelta* × *polonicum* will be given in Table 9. Besides the percentage of seed setting directly taken from his

TABLE 9. *Seed setting in F_1 plants of Spelta × polonicum* (MATSUMURA, *1936 a*).

Year of crossing	*Spelta*		*Polonicum*		F_1, S ♀ × P ♂		F_1, P ♀ × S ♂	
	Grains per head	Seed setting %	Grains per head	Seed setting %	Grains per head	Seed setting %	Grains per head	Seed setting %
1933, free ..	28,4	94,03	46,6	88,58	—	—	—	—
1933, isolated	—	—	—	—	—	—	18,9	44,01
1934, free ..	28,7	91,40	41,6	90,63	27,0	56,72	30,5	64,07
1934, isolated	30,0	90,90	39,5	82,29	21,8	51,17	23,5	53,34
Average	29,0	92,11	42,6	87,17	24,4	53,95	24,3	53,81

table, I have also calculated and included in Table 9 the corresponding figures of the number of grains per head.

The seed-setting figures given by MATSUMURA refer to the first and second flower in each spikelet, while my own figures in Tables 6—8 have reference to all flowers of the spikelets. MATSUMURA's mode of calculation gives a higher value, especially for the hexaploid parents. The number of grains per head is strikingly close to my own figures. For the hexaploid parent MATSUMURA's figure is 29,0 and mine 30,0 (Table 6), for the tetraploid parent the figures are 42,6 and 49,1, and for F_1 24,3 and 24,5 respectively. It is also interesting to note the decreased seed setting after isolation in comparison with free flowering in MATSUMURA's material.

The only cytological investigations carried out in pentaploid F_1 by the present author are chromosome counts in root tips of eight plants belonging to F_1 of the crosses Nos. *66*, *67*, and *69* of *vulgare* × *turgidum*, which all had the expected $2n = 35$ except one plant of cross *69* with $2n = 34$. In hexaploid F_1 some plants of the crosses Nos. *68* and *75* (SV × S and SV × SVT) proved to have $2n = 42$ as expected.

6. THE DISTRIBUTION OF CHROMOSOME NUMBERS IN F_2 AND SUBSEQUENT GENERATIONS.

The chromosomal conditions in F_2 of pentaploid wheat crosses have already been outlined on page 278. The chromosome numbers

have been counted in F_2 by many authors, but only in the combinations *Spelta* × *polonicum*, *vulgare* × *durum*, and *compactum* × *persicum* has the number of individuals been sufficiently comprehensive to give a relatively reliable picture of the frequencies. These three counts, which were all carried out by MATSUMURA (1936 a, 1939), are given in Table 10 and Fig. 3. Here the counts performed by the present author on root tips of F_2 plants of *vulgare* × *turgidum* are also recorded. The F_2 plants belonging to this investigation were taken from four different crosses, Nos. *8*, *67*, *69*, and *77*, as seen in Table 11.

The distribution of the F_2 chromosome numbers in *vulgare* × *turgidum* is apparently analogous in essentials to the distributions found

TABLE 10. *The frequency of different chromosome numbers in pentaploid F_2.*

Species combination 6x × 4x	Chromosome numbers															Total	Author
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42		
Spelta × *polonicum*	16	18	36	37	47	50	48	67	81	77	75	60	42	16	5	675	MATSUMURA, 1936 a
vulgare × *durum*	6	16	6	10	9	9	9	7	10	14	9	6	3	2	—	116	MATSUMURA, 1939
compactum × *persicum*	—	2	8	10	7	8	14	7	13	10	17	7	3	1	—	107	MATSUMURA, 1939
vulgare × *turgidum*	4	9	14	16	9	16	8	11	9	10	11	12	2	1	2	134	GRANHALL, (present paper)

by MATSUMURA in other pentaploid wheat crosses. In comparison with the theoretical distribution, if all the numbers of the gametes are formed at random and freely combined, the most striking deviations of the actual frequencies in MATSUMURA's material are the over-representation of the lower numbers 28—31 and the under-representation of the intermediate numbers 34—36. The reasons are summarized by KIHARA and MATSUMURA (1940) — on the basis of extensive investigations in F_2 and back-crosses — as follows: (1) from the fertility of the selfing of the F_1 hybrid it is evident that only about half of the embryo sacs are fertilized, and that especially the embryo sacs with intermediate chromosome numbers are left unfertilized, (2) the univalents are not distributed at random but partly in groups; the euploid 14- and 21-gametes are thus more frequent than expected, (3) the elimination of univalents causes the production of numerous low-chromosomal gametes and F_2

plants, (4) the 14- and 21-chromosome pollen-grains play the most important rôle at the fertilization, and the pollen-grains with higher numbers are more active than those with lower numbers, (5) the endosperm development in zygotes from low-chromosomal egg-cells and high-chromosomal male gametes is defective, which causes the destruction of many zygotes with $\pm$ 35 chromosomes and 3 $(AB) + (\pm C)$ endosperms, (6) the zygotic elimination of sterile chromosome combinations

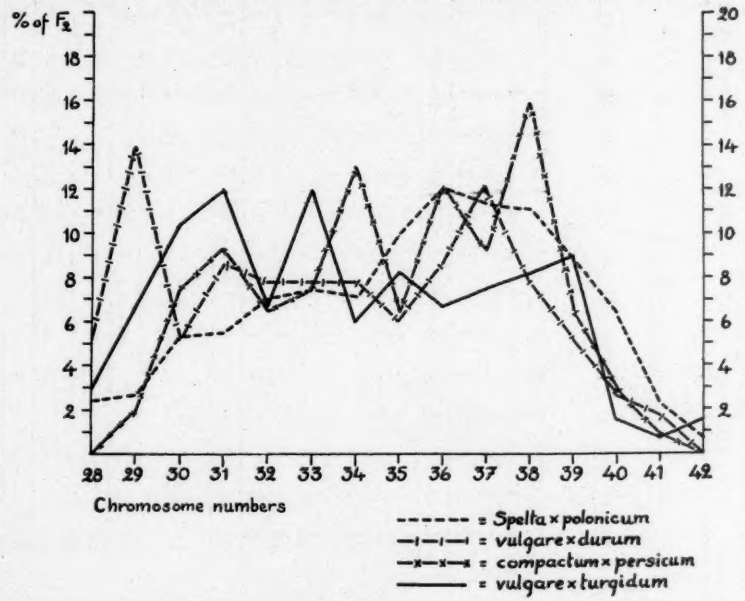

Fig. 3. The distribution of different chromosome numbers in F_2 of hexaploid $\times$ tetraploid *Triticum* (cf. Table 10).

especially falls on the intermediate numbers, and (7) in the decreasing group the incomplete single *C*-genome disturbs the germination and vitality of the low-chromosomal embryos.

In general the processes numbered (3)—(5) above have the most important influence upon the frequencies of different chromosome numbers in the F_2 plants. KIHARA and MATSUMURA, however, also found that the processes play a somewhat different part in the different species crosses. Thus the elimination of univalents (3) is stronger in *vulgare* $\times$ *durum* than in *Spelta* $\times$ *polonicum* and the low-chromosomal frequencies consequently higher in the former cross. In *Spelta* $\times$ *polonicum*

TABLE 11. *Chromosome numbers and morphological type in different crosses and generations.*

(In F_3 and subsequent generations the plants are mainly selected for constant type; not taken out at random. See text.)

Cross No.	Combin.	Type of parent plants	Plants counted in	Chromosome numbers 28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	Total
8	V × T	v/t	F_2	—	—	—	3	5	4	3	4	4	3	1	1	1	—	2	—	—	—	31
67	»	»	»	1	4	4	5	3	3	1	2	2	—	2	4	—	—	—	—	—	—	31
69	»	»	»	1	2	6	3	—	5	3	4	1	5	2	2	1	—	—	—	—	—	35
77	»	»	»	2	3	4	5	1	4	1	1	2	2	6	5	—	1	—	—	—	—	37
				4	9	14	16	9	16	8	11	9	10	11	12	1	1	2	—	—	—	134
66, 67	V × T	v	F_3	1	3	—	3	—	4	2	—	2	3	5	2	7	9	1	—	—	—	42
» »	»	s	»	—	2	1	—	1	—	3	3	3	1	3	6	9	4	2	—	5	—	43
» »	»	v/s	»	—	—	—	1	2	1	1	1	1	1	2	4	1	—	1	1	—	—	17
» »	»	t	»	70	11	1	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	84
» »	»	tc	»	18	5	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	24
» »	»	div.	»	5	5	1	2	2	—	—	1	1	2	3	—	2	1	—	—	—	—	25
				94	26	3	6	5	6	7	5	8	7	13	12	19	14	4	1	5	—	235
5–11, 67	V × T	v	F_4	—	—	—	—	—	—	—	—	—	—	1	2	3	10	9	—	—	—	25
»	»	s	»	—	—	—	—	—	—	—	—	—	—	1	2	6	8	18	10	36	4	85
»	»	v/s	»	—	—	—	—	—	—	—	1	1	—	—	1	1	3	2	—	—	—	9
»	»	v/t	»	—	—	—	—	1	—	—	—	1	—	—	—	2	—	—	—	—	—	4
»	»	t	»	60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	60
»	»	tc	»	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
				64	—	—	—	1	—	—	1	2	—	2	5	12	21	29	10	36	4	187
1	V × T	v	F_5	—	—	—	—	—	—	—	—	—	—	—	1	9	17	36	—	—	—	63
»	»	s	»	—	—	—	—	—	—	—	—	—	—	—	—	4	16	29	1	—	—	50
»	»	v/t	»	—	—	—	—	—	—	—	—	—	2	1	1	5	1	1	—	—	—	11
»	»	t	»	52	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	52
»	»	tc	»	23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	23

»	»	v/t	»	—	—	—	—	—	—	—	—	—	2	1	1	4 5	16 1	29 1	1 —	—	—	50 11
»	»	t	»	52	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	52
»	»	tc	»	23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	23
				75	—	—	—	—	—	—	—	—	2	1	2	18	34	66	1	—	—	199
1	V × T	v	F_9	—	—	—	—	—	—	—	—	—	—	—	—	—	1	27	—	—	—	28
»	»	s	»	—	—	—	—	—	—	—	—	—	—	—	—	—	2	28	—	—	—	30
»	»	t	»	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33
»	»	tc	»	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14
				47	—	—	—	—	—	—	—	—	—	—	—	—	3	55	—	—	—	105
16, 18, 19, 20, 22	(V × T) × V	v	F_4	—	—	—	—	—	—	—	—	—	—	—	—	—	1	9	—	—	—	10
	»	s	»	—	—	—	—	—	—	—	—	—	—	—	—	—	1	5	—	—	—	6
	»	v/s	»	—	—	—	—	—	—	—	—	—	—	—	—	1	—	2	—	—	—	3
				—	—	—	—	—	—	—	—	—	—	—	—	1	2	16	—	—	—	19
17, 21, 23	(V × T) × T	v	F_4	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	3
»	»	s	»	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	2
»	»	t	»	24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24
»	»	tc	»	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
				26	—	—	—	—	—	—	—	—	—	—	—	—	2	3	—	—	—	31
24—26	V × VVT	v	F_4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	6
»	»	v/s	»	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	2
				—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	—	—	—	8
30	S × SVT	s	F_4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	2
28	T × TVT	t	F_4	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8
																						928

Abbreviations: v = *vulgare*-like, s = *Spelta*-like, v/s = intermediate *vulgare-Spelta*, v/t = intermediate F_1-like, t = *turgidum*-like, tc = »*turgidum-compactum*-like» (*contractum*).

the relatively high activity of the pollen-grains with higher numbers (4) causes an obvious increase of the higher F_2 numbers, which is not so pronounced in *vulgare* × *durum*. The elimination of sterile intermediate combinations (6) is especially intense in *vulgare* × *durum*, where the curve is even bimodal. The elimination of low-chromosomal embryos (7) is, finally, most evident in *Spelta* × *polonicum*.

The frequency curve of *vulgare* × *turgidum* is, just as that of *compositum* × *persicum*, somewhat irregular, which indicates that a greater number of individuals would have been desirable. It seems, however, justifiable to draw certain conclusions from the distribution observed. Thus the over-representation of the low numbers 28—31 is very conspicuous, which makes an intense elimination of univalents as in *vulgare* × *durum* probable. The depression of the intermediate frequencies and the indication of double peaks also recall the *vulgare* × *durum* distribution, caused *inter alia* by intense elimination of sterile combinations.

Chromosome counts were also made for F_3 and subsequent generations by the present author in a number of plants belonging to the 6000-, 8000-, 9000-, and 0000-series. As described on page 272, the »lines» of these series have been selected for constancy of type and also of fertility and vitality in order to obtain tetraploid and hexaploid lines for the freezing trials. In Table 11 all these counts are collected and grouped according to the morphology of the parent plants. On account of the conscious selection the chromosome frequencies in this table do not represent real samples of the variation in different generations, and the table cannot therefore be used for close comparisons. It is evident, however, that the variation is great in F_3 even in the progenies of selected F_2 plants, and it is also evident that this variation in the chromosome numbers is far greater in the »increasing group» than in the »decreasing group».

In F_4 it already seems simple to obtain constant 28-chromosomal lines from selected F_3 plants, but the same stabilization is still not reached on the high-chromosomal side of the table. Also in F_5 there is often a variation between 40 and 42 in the »increasing group». In the generations F_6—F_8 no counts were performed, but in F_9 the tests showed that as a rule the euploid stage was at last attained among the *vulgare*- and *Spelta*-like lines.

In F_7 of *vulgare* × *durum* VAKAR, KROT, and BREKINA (1932) observed irregularities in the pollen-grain formation of 28- and 42-chromosomal lines, and they also refer to similar results obtained by

A. A. SAPEHIN in 42-chromosomal F_7 plants of the same species cross, while L. A. SAPEHIN, who investigated the corresponding 28-chromosomal plants, found a more regular meiosis. LOVE (1940), studying F_5—F_8 of the same species constellation, found that cytological aberrations in the shape of inversions, translocations, deficiencies, and duplications were very common, which means that it takes a considerable time to reach chromosomal balance in the pentaploid wheat crosses. It is also interesting to note the statement of POWERS (1932) that the variety Marquillo ($2n = 42$), derived from the cross in 1914 between *vulgare* Marquis and *durum* Iumillo, shows a high percentage of chromosomal abnormalities. As a conclusion it has been emphasized by several investigators, dealing with pentaploid wheat crosses (cf. KIHARA and MATSUMURA, 1940), that the constant 28-chromosomal stage is rapidly reached, whereas the 42-chromosomal stage is not completely attained until after a long series of generations. My own experiences from the repeated selection for constant type clearly agree with this general rule.

According to KIHARA's scheme, no other constant chromosome numbers result than those of the parents, viz. 28 and 42. Exceptions are, however, found. Thus KIHARA himself (1924) reports the occurrence of two constant 40-chromosomal dwarf plants in *Spelta* × *polonicum*. SAPEHIN and SAPEHIN (1928) have described a 36-chromosomal line out of *vulgare* × *durum* which had been constant for a long course of years, although the original, quite fertile plant with 16_{II} and 4_I should formally belong to the »sterile combinations». According to KIHARA and MATSUMURA (1940), however, this finding has later proved to be wrong.

In my own material there is one case of relative constancy in chromosome numbers outside the normal numbers 28 and 42 to be recorded. In F_3 of the cross No. 67 (Minhardi × Rivet Cambr. 4) five *speltoides* plants representing the plot 501—53 (= 0053) all proved to have $2n = 44$. This plot was derived from a single F_2 plant which had the typical *speltoides* habitus.

In F_4 57 plants, derived from ten different F_3 plants of this line (0053), were counted for their somatic numbers, as seen in Table 12. Originally six grains were sown from each one of the selected ten F_3 plants of 0053, and they all germinated with the exception of three grains from the F_3 plant No. 9. In all, the counts of F_4 gave 1—39, 2—40, 4—42, 10—43, 36—44, and 4—45. As for the different F_3 plants, eight of them gave a progeny with merely or mostly 44, one gave 42—43,

TABLE 12. *Chromosome numbers in F_4 of line 0053.*

F_3 plant	Chromosome numbers in F_4, plant No.					
	1	2	3	4	5	6
No. 501—53—1	44	44	44	45	44	44
2	44	44	44	44	43	44
3	44	43	45	45	44	44
4	43	44	44	44	44	44
5	43	44	44	43	43	44
6	44	44	44	44	44	44
7	43	43	42	42	43	42
8	44	44	44	44	45	44
9	40	39	40	—	—	—
10	44	44	42	44	44	43

and, finally, one, namely 501—53—9, gave 39—40. The latter F_3 plant thus completely distinguishes itself from its sister plants by chromosome number and probably also by poor germination, which points to some meiotic irregularities. As a whole, however, the line 0053 has mainly $2n = 44$ and represents a fully fertile *speltoides* of ordinary growth.

An analogous case of 44-chromosomal aberrants has been reported by NISHIYAMA (1939) in the cross *Avena fatua* ($2n = 42$) × *A. barbata* ($2n = 28$). In the back-cross *Tr. Spelta* ♀ × (*Spelta* × *polonicum*) F_1 ♂ MATSUMURA (1936 b) has also found a 44-chromosomal plant.

Among other unexpected numbers found in the progenies of *vulgare* × *turgidum* a diploid plant with $2n = 14$ may also be mentioned. This plant was found in F_3 of the line 0062 in the cross No. 67 (Minhardi × Rivet Cambr. 4). Four sister plants, derived from the same *turgidum*-like F_2 plant, all had $2n = 28$. In the same cross a single chromosome plate with $2n = 58$ in an otherwise 29-chromosomal plant of 0042 was also observed.

In pentaploid *Avena* crosses NISHIYAMA (1939) also found a 14-chromosome plant, which presents another parallel to my own findings in pentaploid *Triticum*.

In F_4 of the back-cross (*vulgare* × *turgidum*) F_1 ♀ × *vulgare* ♂ all the selected plants in my material had 42 chromosomes, or nearly so. In the same generation of the back-cross with the other parent (F_1 ♀ × *turgidum* ♂) most plants had 28 chromosomes, but five plants of *vulgare*- or *speltoides*-type and 41—42 chromosomes were also selected. The origin of these deviating plants, which belonged to the lines 9019, 9021, and 9041, goes back to three different F_1 plants of the crosses

Nos. *17* and *21*. If a male gamete with 14 chromosomes from *turgidum* unites with a female gamete with 21 from (V × T) F_1, 35 can be obtained, and entry into the »increasing group» is possible.

In MATSUMURA's (1939) counts of the back-cross (*vulgare* × *durum*) F_1 ♀ × *durum* ♂ only 28—34 and no case of 35 appeared, though this number is theoretically possible. The majority had 28—30, and it is obvious that 35 must in any case be a rare number in this type of back-cross. In (*Spelta* × *polonicum*) F_1 ♀ × *polonicum* ♂ KIHARA and WAKAKUWA (1935) observed a single 38- (and also a 48-) chromosomal plant, which indicates that the increasing type also can arise through irregularities at meiosis directly following the back-crossing. Other examples of unexpected numbers in back-crosses are $2n = 32$ in F_1 ♀ × *Spelta* ♂ (KIHARA and WAKAKUWA, 1935), $2n = 36$ in *polonicum* ♀ × F_1 ♂, and $2n = 44$ in *Spelta* ♀ × F_1 ♂ (MATSUMURA, 1936 b).

Finally, material derived from the F_4 of crosses between extracted 6000-lines and habitually similar species has been counted in a few cases. The results proved very monotonous, the plants from hexaploid crosses had all $2n = 42$, and the plants from tetraploid crosses all $2n = 28$.

7. MENDELIAN INHERITANCE IN PENTAPLOID WHEAT HYBRIDS.

In his classical work »Mendel's Laws of Inheritance and Wheat Breeding», published in 1905, BIFFEN concludes that he has found 3 : 1 ratios for the segregation of some morphological characters in crosses between *int. al. vulgare* and *turgidum*. Among these characters he mentions beardless: bearded ears; pubescent: glabrous glumes; gray: white ears; keeled: rounded glumes; hollow: solid straw; yellow rust susceptibility: resistance. Several qualities and among them also the three last-mentioned pairs have, however, later on proved to segregate in a far more complicated way than supposed by BIFFEN. It is now evident that the mode of segregation depends upon to which genome the genes in question belong. If they are located in the homologous *A*- or *B*-genomes Mendelian segregation can be expected in F_2, if the *C*-genome is involved the segregation is also dependent upon the distribution of univalents, and F_2 will exhibit a very confusing picture with transgressions and other unexpected phenomena (cf. WATKINS, 1930). In this last-mentioned case the factorial analysis cannot be carried out in F_2 but in back-crosses of F_1 to the parents. The egg-cells of F_1 are practically all fertile in such back-crosses, but at self-fertilization the

sterility of the F_1 pollen and the elimination and unequal distribution of univalents will disturb the factorial combination. If, on the other hand, the F_1 egg-cells are fertilized by parental pollen, the fertility will be without objections.

Factorial analysis has not been performed in my material, with the exception of a number of F_2 segregations as to the characters beardness and pubescence of the heads. The agreement between the frequencies found and the Mendelian expectations has been tested statistically by means of the χ^2 method.

In eight pentaploid crosses simultaneous segregation of these two characters has been procured in F_2, as seen in Table 13.

TABLE 13. *Segregation of beardness and pubescence in F_2.*

Cross No.	Pubescent, Beardless	Pubescent, Bearded	Glabrous, Beardless	Glabrous, Bearded	Total
1	64	22	21	4	111
5	390	175	152	90	807
6	395	151	145	54	745
7	306	110	105	33	554
10	190	80	81	28	379
11	357	130	116	35	638
59	35	19	9	12	75
82	17	5	16	1	39
Total	1754	692	645	257	3348

The χ^2 analysis according to the agreement with the assumptions of 3 : 1 and free recombination will give the following results (Table 14).

The following conclusions can be drawn from this analysis. Cross No. *5* shows disagreement with the expectation as to all the three assumptions, cross No. *59* as to the beardness, and cross No. *82* as to the pubescence and the free combination. A closer investigation showed that the parent TcVT 6153 in cross *59* was partly heterozygous for beardness, which explains its deviation as to this character. In cross *82* the number of individuals is too small. In cross *5* (Sol III $\times$ Compositum), however, the disagreements could also be verified in F_3, where eight families segregating for pubescence together gave a quotient of 182 pubescent : 86 glabrous ($\chi^2 = 7{,}184^{**}$), and nine families segregating for beardness showed 187 beardless : 82 bearded plants ($\chi^2 = 4{,}314^{*}$). In a back-cross between F_1 of cross *5* to the *vulgare* parent Sol III, named cross *16*, where the ratios 1 *BB Pp* : 1 *Bb Pp* : 1 *BB pp* : 1 *Bb pp* (*B* = beardless, *P* = pubescent) should be expected, the actual dis-

tribution was 3 : 2 : 4 : 0. In the other back-cross No. *17*, where F_1 was crossed with *turgidum* Red Rivet, the expectation was 1 *Bb PP* : 1 *Bb Pp* : 1 *bb Pp* : 1 *bb pp* and the actual values 3 : 2 : 1 : 0. The material in these back-crosses is far too little to admit of any conclusions. In the segregating families after self-fertilization of the back-cross (V×T)×V, the quotients 100 beardless : 25 bearded and 203 pubescent : 62 glabrous were obtained (cross *16*, F_2), and the corresponding figures from the back-cross (V × T) × T were 105 beardless : 36 bearded and 61 pubescent : 19 glabrous (cross *17*, F_2), which means quite normal 3 : 1 ratios ($\chi^2 = 1{,}6800^{0(0)}$, $0{,}3635^{000}$, $0{,}0213^{000}$, $0{,}0667^{00}$ respectively). The question whether the segregation of pubescence and beardness in F_2 of V × T in cross No. *5* has been influenced by the irregular chromosomal conditions and the sterility of the pollen-grains is consequently not answered, although it seems as if more normal ratios have been obtained at the self-fertilization of the back-cross, where the chromosome variation is more limited. In any case it is interesting to note that the two crosses *5* and *82* both have Compositum as the *turgidum* parent. All the other crosses in which Red Rivet is involved behave in agreement with the Mendelian ratios and the free recombination in F_2.

TABLE 14. χ^2 *analysis of the* F_2 *segregation.*

Cross No.	Agreement with 3 : 1 as to Pubescent : Glabrous	Agreement with 3 : 1 as to Beardless : Bearded	Agreement with free recombination	Total χ^2	D. F.
1	$0{,}3634^{000}$	$0{,}1471^{000}$	$0{,}8414^{000}$	$1{,}3523^{000}$	3
5	$11{,}7972^{***}$	$26{,}4391^{***}$	$6{,}6035^{*(*)}$	$44{,}8398^{***}$	3
6	$1{,}1638^{000}$	$2{,}5168^{00}$	$0{,}0073^{000}$	$3{,}6879^{000}$	3
7	$0{,}0024^{000}$	$0{,}1949^{000}$	$0{,}3538^{000}$	$0{,}5511^{000}$	3
10	$2{,}8575^{0}$	$2{,}4705^{00}$	$0{,}4928^{000}$	$5{,}8208^{00}$	3
11	$0{,}6046^{000}$	$0{,}2529^{000}$	$0{,}7586^{000}$	$1{,}6155^{000}$	3
59	$0{,}3600^{000}$	$10{,}6711^{**(*)}$	$1{,}8148^{00}$	$12{,}8459^{**}$	3
82	$7{,}1880^{**}$	$1{,}9231^{00}$	$3{,}9002^{*}$	$13{,}0113^{**}$	3
Total	$24{,}3363^{**}$	$44{,}6155^{***}$	$14{,}7728^{0}$	$83{,}7246^{***}$	24
χ^2 for the total	$6{,}7304^{**}$	$19{,}9825^{***}$	$0{,}1041^{000}$	$26{,}8170^{***}$	3
Heterogeneity	$17{,}6059^{*}$	$24{,}6330^{***}$	$14{,}6687^{*}$	$56{,}9076^{***}$	
D. F.	7	7	7	21	

If the crosses where only one of the two qualities pubescence and beardness are segregating, are considered, some further observations can be added. As to the pubescence, the segregation in one tetraploid

and two hexaploid crosses has also been counted as follows (Table 15). The results are in full agreement with expectation.

TABLE 15. *F_2 segregation of pubescence.*

Cross		Pubescent	Glabrous	Total	Agreement with 3 : 1, χ^2
Tetraploid T × TcVT	*57*	303	101	404	$0{,}0000^{000}$
Hexaploid S × SVT	*45*	683	222	905	$0{,}1064^{000}$
» V × C	*83*	2047	658	2705	$0{,}6567^{000}$

Solely beardness has been studied in a series of crosses, of which one is tetraploid, two are pentaploid in addition to the eight of the »double» table, and nine are hexaploid. The agreement is not quite in order in the case of four of the crosses in Table 16, namely in Nos. *8* (Pudel × Red Rivet), *9* (Gluten × Red Rivet), *2* (Kron × Bearded Spelt), and *15* (Pansar × Bearded Pansar). In the two first crosses, both pentaploid, too many bearded plants are counted; in the other two crosses, which are hexaploid, the bearded plants are under-represented. It is especially remarkable that the cross between beardless and bearded Pansar shows deviation in spite of the great number of individuals. The significance of all the four deviations is, however, relatively weak. The F_3 of the two crosses Nos. *8* and *15* has not been investigated. In cross No. *9* two segregating F_3 families gave together 47 beardless : 17 bearded, which is quite normal. Also in the cross No. 2, where F_3 has been more thoroughly studied, the segregation seemed to be in

TABLE 16. *F_2 segregation of beardness.*

Cross		Beardless	Bearded	Total	Agreement with 3 : 1, χ^2
Tetraploid T × TVT	*57*	292	112	404	$1{,}5974^{000}$
Pentaploid V × T	*8*	422	177	599	6,6116*
» »	*9*	403	164	567	4,6567*
Hexaploid V × S	*2*	172	37	209	5,9346*
» »	*4*	60	12	72	$2{,}6667^{0(0)}$
» V × V	*15*	1254	367	1621	4,8137*
» S × SVT	*43*	349	124	473	$0{,}3728^{000}$
» »	*48*	332	105	437	$0{,}2204^{000}$
» »	*50*	333	115	448	$0{,}1071^{000}$
» »	*52*	146	44	190	$0{,}3439^{000}$
» »	*53*	104	29	133	$0{,}7243^{000}$
» S × SV	*68*	374	144	518	$2{,}1647^{00}$

agreement with 3 : 1, 873 beardless and 297 bearded plants being found in the segregating families.

8. CHROMOSOME NUMBER AND MORPHOLOGICAL CHARACTERS.

The association between chromosome number and morphological type is usually very evident in the progenies of pentaploid wheat. KIHARA (1924) expresses this fact in the following words: »Der Habitus der 28-chromosomigen Nachkommen dieser pentaploiden Weizenbastarde ähnelt meistens mehr oder weniger dem Emmertypus, während die 42-chromosomigen typischen oder annähernd typischen Dinkelhabitus besitzen». A closer examination, however, has shown that several typical »dinkel characters» can be transferred to 28-chromosomal progenies and »emmer characters» to 42-chromosomal lines, but also that this transference succeeds more or less easily and more or less completely in different characters. (1) In the tetraploid wheat species it is, for instance, extremely rare to find beardless varieties, while such types are very common among the hexaploid species. When, however, a bearded tetraploid is crossed with a beardless hexaploid, beardless tetraploids arise in the progeny by regular pairing of the homologous (*B*-)genomes. (2) Respecting another complex of characters comprising the waxiness of the leaves, the development of the glume keel (see p. 310), and the resistance to yellow rust, etc., WATKINS *et al.* (1927 a, b, 1931, 1940) and others have proved that inheritance of these is governed by genes located both in the emmer genomes *AB* and in the dinkel genome *C*. The transferences between the hexaploid and the tetraploid stages are possible also here but in lower frequencies. (3) Finally, there are also a number of characters which are determined only or mainly by genes of the *C*-set. In this case exchange must be very rare and can only be explained by occasional crossing over between non-homologous genomes. PETERSON and LOVE (1940) have given some interesting results concerning the inheritance of stem rust (*Puccinia graminis*) immunity in *vulgare* × *durum*, which seem to indicate that the *C*-genome of hexaploid progenies contains genes that inhibit or reduce the effects of the genes for immunity transferred to the *AB*-genomes from *durum*. The possibility of obtaining fully immune hexaploids by crossing over is, however, indicated by observations on multivalents and translocations in F_1. The investigations by YAMASHITA (1937) on the solidity of the straw in different pentaploid crosses also suggest a dominant factor in the *C*-genome as a reason for the special hollow dinkel type. In the material of THOMPSON, ARNASON, and LOVE (1935) no 28-chromosomal lines of *vulgare* × *durum* or *vulgare* × *dicoccum* had the thin-walled stems characteristic of *vulgare*, which is explained in a similar way.

At the type classification of the different progenies from pentaploid wheat crosses characters belonging to the groups (2) and (3) mentioned above are consequently especially useful, and special attention was

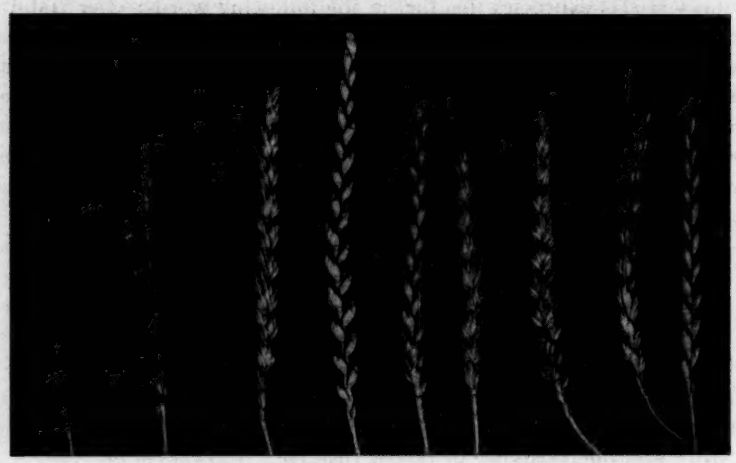

Fig. 4. Heads of seven *speltoides* types (to the left) from the 6000-series (cross No. *1*) compared with two varieties of *Spelta* (to the right).

also given to such characters in my investigations. Four different main types were separated, namely the two parental types, *vulgare*- and *turgidum*-like, and the forms *speltoides* and *contractum*. The

Fig. 5. Heads of six *contractum* types (to the left) from the 6000-series (cross No. *1*) compared with three varieties of *compactum* (to the right).

nomenclature of the last-mentioned types has been borrowed from KAJANUS (1923), who has also given adequate descriptions of them (1923, p. 135). *Speltoides* (Fig. 4) is strongly reminiscent of *Tr. Spelta*,

with long, hollow straw; lax ears; keeled, truncate, and firm empty glumes; but with comparatively tough rachis in opposition to *Spelta*, which has a very fragile rachis. *Contractum* (Fig. 5) recalls *Tr. compactum*, with short straw, usually curved just under the head; extremely dense ears; round, loose empty glumes, keeled only at the top. It differs, however, from *compactum* with regard to the *turgidum*-like apical tooth of the empty glume and the solid or nearly solid straw. KAJANUS assigns the *contractum* forms to *turgidum*, while several other investigators (e. g. RAUM, 1931, 1934) have characterized them as real *compactum*. As shown by WATKINS (e. g. 1930), they are tetraploid and are closely connected with *turgidum*. In the *vulgare*-like group I have also included KAJANUS's »*lanceolatum*-type» with long glumes, and to the *turgidum*-like group I have referred a number of *durum*-like plants with flat ears. The *lanceolatum*- and the *durum*-types are both highly sterile and they therefore soon disappear in later generations.

In Tables 17—20 the origin, chromosome numbers, and morphological type have been recorded for the lines belonging to my 6000-, 8000-, 9000-, and 0000-series. Some brief remarks concerning the correlation between chromosome number and type may be given here.

In the 6000-series (Table 17) 87 lines were counted in F_5. The hexaploid lines ($2n = 39$—43) were all *vulgare*-like or *speltoides*, 28 of the former and 20 of the latter type. Two lines with more intermediate numbers ($2n = 37$—40) proved to be segregating in type but mostly resembled *vulgare*. Among the tetraploid lines, which all had the euploid number 28, 25 were *turgidum*-like, but the other 12 mostly resembled *compactum* and were identified with KAJANUS's *contractum*-type. As seen in the table, lines derived from the same F_3 plot and F_2 plant have corresponding chromosome numbers, but may be different in type within the same group, for instance, line 6016 *vulgare*-like and 6017 *speltoides*.

The 8000-series (Table 18) is derived from the crosses Nos. *5—11*, all V × T. The 75 lines counted in F_4 comprise 56 hexaploids ($2n = 38$—42), partly *vulgare*, partly *speltoides*, and partly segregating in these two types; 3 pentaploids ($2n = 32$—40), segregating like F_2; 36 tetraploids ($2n = 28$), 3 of which were *contractum* and the others *turgidum*-like. The different lines in this series usually go back to different F_2 plants; No. 8070, however, is pentaploid and its sister line 8071 hexaploid; No. 8081 is *vulgare*-like and 8082 is *speltoides*, but these discrepancies are not astonishing.

TABLE 17. *The 6000-series, origin, chromosome number, and type.*

1934 F_3 954* 955-	1935 F_4 366-	1936 F_5 Line	1936 2n	1936 type	1934 F_3 955-	1935 F_4 366-	1936 F_5 line	1936 2n	1936 type
6*	12	6009	42	v	42	104	6159	28	c
»*	»	6010	42	v	»	»	6160	28	c
»*	»	6011	42	v	»	»	6162	28	c
8*	26	6016	41—42	v	44	105	6163	41—43	s
»*	»	6017	41—42	s	»	»	6165	42	s
17*	31	6019	40—42	s	»	»	6166	41	s
»*	»	6020	40—42	s	»	»	6167	40—42	s
»*	»	6021	42	s	51	107	6172	41—42	s
»*	32	6024	40—41	s	»	»	6173	42	s
»*	»	6025	41—42	s	»	108	6174	42	s
»*	»	6026	41—42	s	»	»	6175	42	s
1	44	6038	28	c	»	»	6176	42	s
»	45	6039	28	c	»	»	6177	42	s
»	»	6041	28	c	»	110	6179	42	s
»	»	6043	28	c	»	»	6180	42	s
3	47	6048	42	v	»	»	6183	42	s
»	»	6049	41—42	v	54	114	6191	28	t
»	»	6050	42	v	»	»	6193	28	t
»	48	6054	42	v	»	»	6194	28	t
»	»	6058	42	v	»	»	6195	28	t
»	»	6064	42	v	63	118	6202	28	t
»	49	6068	42	v	»	»	6203	28	t
»	»	6071	42	v	»	»	6204	28	t
»	»	6072	41—42	v	»	»	6206	28	t
»	50	6074	41	v	65	123	6207	28	t
»	53	6086	42	v	»	»	6208	28	t
4	55	6088	28	t	68	132	6215	28	t
»	»	6090	28	t	»	»	6216	28	t
»	»	6093	28	t	»	»	6217	28	t
6	57	6098	41	v	76	134	6221	28	c
7	62	6103	40—41	v	80	135	6223	28	t
»	»	6104	40	v	»	»	6224	28	t
29	86	6136	28	t	»	»	6225	28	t
»	»	6138	28	t	90	138	6227	38—40	v/t
»	»	6139	28	t	»	»	6228	37	v/t
»	»	6140	28	t	98	139	6229	42	v
»	88	6142	28	t	»	»	6231	41	v
30	90	6145	28	t	109	144	6240	41—42	v
32	93	6146	40	v	»	»	6241	41	v
»	»	6147	39—41	v	»	»	6244	41—42	v
»	»	6148	40	v					
40	97	6149	39—42	v					
»	98	6150	40	v					
41	103	6153	28	c					
»	»	6155	28	c					
»	»	6157	28	c					
42	104	6158	28	c					

v = *vulgare*-like	28
s = *speltoides*	20
v/t = segregating	2
t = *turgidum*-like	25
c = *contractum*	12
Total	87

TABLE 18. *The 8000-series, origin, chromosome number, and type.*

Cross No.	1937 F_3 261-	1938 F_4 line	1938 2n	1938 type	Cross No.	1937 F_3 261-	1938 F_4 line	1938 2n	1938 type
5	14	8005	28	c	7	84	8050	28	t
»	»	8006	28	c	»	87	8051	35—36	s/t
»	15	8009	28	t	»	88	8052	40	v/s
»	22	8012	41	v	»	90	8053	39	s
»	25	8014	28	t	»	91	8055	42	s
»	27	8016	28	t	»	92	8056	28	t
»	28	8017	36—40	s/t	»	»	8057	28	t
»	32	8018	28	t	*8*	96	8061	39	v/s
»	33	8020	41	v	»	99	8062	41—42	v
»	36	8022	41	v	»	102	8064	40—42	v
»	38	8026	28	c	*9*	115	8068	28	t
»	40	8027	28	t	»	117	8069	28	t
»	42	8028	28	t	»	119	8070	32	v/t
»	45	8029	42	v	»	»	8071	40	v
»	47	8030	40	s	»	121	8072	28	t
»	49	8031	42	v	»	123	8073	41	v
»	181	8104	28	t	»	126	8074	28	t
»	196	8106	28	t	»	128	8075	28	t
»	201	8107	28	t	»	132	8076	28	t
»	205	8108	28	t	»	133	8077	41—42	s
»	217	8109	41—42	s	*10*	141	8081	38—39	v
»	218	8110	28	t	»	»	8082	40—41	s
»	221	8112	42	v	»	142	8083	28	t
»	233	8115	28	t	»	»	8084	28	t
»	238	8117	28	t	»	143	8085	39—42	v
»	243	8118	28	t	»	145	8086	40—42	s
»	247	8120	41	s	»	148	8087	41	s
»	248	8122	28	t	*11*	158	8092	42	s
»	249	8123	41—42	v	»	159	8093	42	s
»	251	8125	42	s	»	161	8094	41—42	v
»	252	8127	41	v	»	166	8096	28	t
6	58	8035	28	t	»	167	8098	38—41	s
»	61	8036	40	v	»	168	8099	28	t
»	62	8037	42	s					
»	66	8038	28	t					
»	»	8039	28	t					
»	68	8040	41—42	v					
»	69	8041	42	s					
»	75	8042	42	s					
7	83	8046	41	v/s					
»	85	8047	28	t					
»	»	8048	28	t					

v = *vulgare*-like	17
v/s = segregating	3
s = *speltoides*	16
v/t = segregating	1
s/t = segregating	2
t = *turgidum*-like	33
c = *contractum*	3
Total	75

The 9000-series (Table 19) can be divided into two parts, *A* derived from the back-crosses Nos. *16—23*, and *B* from the crosses Nos. *24—26*, *28*, *30* between 6000-lines and similar species. Of 9000 *A*, 26 lines are counted in F_4 and of 9000 *B* 10 lines. The hexaploid lines (2n = 40—42) are *vulgare*-like, *speltoides* or segregating in these two types; the tetraploid lines (2n = 28) are *turgidum*-like or *contractum*. All lines derive from different F_2 plants.

TABLE 19. *The 9000-series, origin, chromosome number, and type.*

	A				*B*				
Cross No.	1938 F_3 250-	1939 F_4 line	1939 2n	1939 type	Cross No.	1938 F_3 251-	1939 F_4 line	1939 2n	1939 type
16	9	9008	42	v	*24*	4	9070	42	v
»	17	9010	42	v	*25*	33	9081	42	v
17	35	9014	28	t	*26*	37	9086	42	v
»	40	9017	28	t	»	45	9092	42	v/s
»	43	9019	41—42	s	*28*	66	9105	28	t
»	45	9020	28	t	»	71	9109	28	t
»	46	9021	41	v	»	83	9112	28	t
»	48	9022	28	t	»	88	9114	28	t
»	50	9023	28	t	*30*	109	9126	42	s
18	53	9025	41—42	s	»	117	9130	42	s
»	54	9026	42	v					
»	57	9027	42	v					
»	60	9029	42	v/s					
19	62	9031	40	v/s					
20	66	9034	41—42	v					
21	71	9039	28	c					
»	74	9041	42	v					
»	77	9042	28	t					
»	84	9044	28	t					
»	92	9047	28	t					
22	94	9049	42	v					
»	102	9054	42	s					
»	107	9059	42	s					
23	115	9061	28	t					
»	117	9062	28	t					
»	118	9063	28	t					
»	119	9064	28	t					

	A	*B*
v = *vulgare*-like	7	3
v/s = segregating	2	1
s = *speltoides*	4	2
t = *turgidum*-like	12	4
c = *contractum*	1	—
Total	26	10

The 0000-series (Table 20), finally, is derived from the crosses Nos. *66* (*A*) and *67* (*B*), both V × T. Chromosome counts were already performed in F_3 in 18 lines from *A* and 28 from *B*. The variation in numbers is greater than in the other series. Altogether 14 lines of both

crosses evidently belonged to the increasing group (2n = 36—44); they were all more or less *vulgare*- or *Spelta*-like. In the case of 10 lines the chromosome numbers varied round the pentaploid stage or nearly over the whole scale (2n = 28—40), and the lines all segregated in type,

TABLE 20. *The 0000-series, origin, chromosome number, and type.*

A				*B*			
Cross No.	1940 F_3 line	1940 2n	1940 type	Cross No.	1940 F_3 line	1940 2n	1940 type
66	0002	33—37	v/t	*67*	0035	28	t
»	0003	37—39	v/s	»	0036	28	c
»	0004	38—40	v	»	0037	28—29	t
»	0006	39—40	s	»	0038	(38)—40	v
»	0007	28—29	c	»	0039	37—40	s
»	0008	41	v	»	0040	29—36	s/t
»	0009	28—29	t	»	0041	34—40	s/t
»	0010	28—29	c	»	0042	28—29 [1]	t
»	0013	41—42	s	»	0043	28	t
»	0015	28	t	»	0044	(36)—40	s
»	0017	40—41	s	»	0045	33—38	v/t
»	0019	28—(33)	c	»	0046	28—29	t
»	0021	28	t	»	0047	34—36	s/t
»	0022	30—36	v/t	»	0048	28—29	c
»	0024	37—41	v	»	0049	40—42	v
»	0025	28	t	»	0050	28—29	t
»	0026	38—39	s	»	0051	28	t
»	0028	28—29	t	»	0053	44 [2]	s
				»	0054	38—43	v/s
		A	*B*	»	0055	28	t
v = *vulgare*-like		3	2	»	0056	28	t
v/s = segregating		1	1	»	0057	28—31	v/t
s = *speltoides*		4	3	»	0058	28	t
v/t = segregating		2	3	»	0059	29—34	v/t
s/t = »		—	5	»	0060	31—36	s/t
t = *turgidum*-like		5	12	»	0061	28	t
c = *contractum*		3	2	»	0062	(14)—28 [3]	t
	Total	18	28	»	0063	34—39	s/t

but the segregation was sometimes more or less limited in the direction of the hexaploid or the tetraploid type. In 22 lines with decidedly tetraploid numbers (2n = 28—29, in line 0019 a single plant with 33) the

[1] = 5 counted; 4—28, 1—29 with a single plate 58.

[2] = 5 counted; all 44 (cf. p. 297).

[3] = 5 counted; 4—28, 1—14.

morphological type was typically *turgidum*-like or *contractum*. Some special aberrations in the chromosome numbers of the 0000 *B*-series have already been reported (pp. 297—298).

9. ON THE OCCURRENCE OF SPELTOIDES AND CONTRACTUM TYPES IN THE PROGENIES OF VULGARE × TURGIDUM.

The occurrence of *speltoides* plants in F_2 and subsequent generations of the cross *vulgare* × *turgidum* was first described by VILMORIN in 1879 (cit. PERCIVAL, 1921, p. 400), and was also observed by BIFFEN (1905) and after him by a great number of other investigators. KAJANUS (1923) draws attention to their resemblance to the speltoids, which sometimes occur in common *vulgare*. His explanation of the origin of the *speltoides* type in *vulgare* × *turgidum* is that it represents the double recessive stage *vvtt* from the two factors *V* from *vulgare* and *T* from *turgidum*. As already mentioned (p. 305), he has also described the *contractum* types, but no scheme of explanation is given in this case. RAUM (1931, 1934), who has carried out extensive morphological studies on the cross *vulgare* × *turgidum*, has given a very detailed factorial scheme in explanation of the origin of the different types. In the absence of cytological control he has, however, made some serious mistakes, presuming the *contractum* types to be hexaploid and the *speltoides* types tetraploid (1934, p. 486), quite contrary to the actual state of things. The most valuable contributions to the solution of these problems are given by WATKINS (1927 b, 1928, 1930, 1940). In his latest publication he presents, *int. al.*, the following formulae for the »*K* complex», regulating the form, firmness, and thickness of the empty glume, the length of the ear internodes, the development of the keel, etc.: Tetraploids—*turgidum* = $KK \,.\, KK$, *dicoccum* = $K^sK^s \,.\, KK$; hexaploids—*vulgare* = $KK \,.\, kk \,.\, K^dK^d$, *Spelta* = $KK \,.\, K^sK^s \,.\, K^dK^d$. (Since most authors seem to agree in the locating of the differences in the »*K* complex» to the genomes *B* and *C*, I have shifted the order of the *K* sets within WATKINS's formulae, e. g. *vulgare* from $kk \,.\, KK \,.\, K^dK^d$ to $KK \,.\, kk \,.\, K^dK^d$, etc.) On the strength of investigations on the degree of linkage between the different components of the *K* complex and the genes B_1 and b_1 (beardless and bearded ears) he concludes that K^s, K^d, *K*, and *k* form a series of multiple allelomorphs (K^s and K^d may be identical), each consisting of a group of completely linked genes. When the two species *vulgare* and *turgidum* are crossed, the new constant constellations 28-chromosomal $KK \,.\, kk$ (= *contractum*) and 42-chromosomal $KK \,.\, KK \,.\, K^dK^d$ (= *speltoides*) finally arise (cf. Fig. 10: I).

The frequency of *speltoides* in F_2 is according to KAJANUS (1923) about 10 %, and according to RAUM (1934) between 0 and 36,11, average 13,23 %. The frequency of *contractum* is not given by these authors.

TABLE 21. *The speltoides and contractum frequencies in* F_2.

Cross No.	Number of F_2 plants	Number of *speltoides*	Number of *contractum*	Frequency % *speltoides*	Frequency % *contractum*
1	112	9	10	8,0	8,9
5	84	5	6	6,0	7,1
6	189	36	—	19,1	—
7	554	47	—	8,5	—
8	599	32	—	5,3	—
9	567	59	—	10,4	—
10	379	62	—	16,4	—
11	638	52	—	8,2	—
14	33	3	—	9,1	—
Total	3155	305	—	—	—
Average	—	—	—	9,67	—
Total	196	14	16	—	—
Average	—	—	—	7,14	8,17

In nine different crosses of V × T I have counted the number of *speltoides* in F_2 and in two of them also the number of *contractum*. As seen from Table 21, the frequency of the *speltoides* has varied between 5,3 and 19,1 % with an average of 9,67 in the whole material. Judging from the two crosses in which *contractum* was also counted, the two new-formed types seem to occur in about the same frequency.

The variation of F_2 gives an impression of continuity, because those character combinations which are typical of the parents are here scattered among its different elements and recombined in different ways. Only in one cross, No. *1*, has a separation of all the four main types, *speltoides*, *vulgare*-like, *turgidum*-like, and *contractum*, been tried in F_2. The segregation thus obtained was 9 : 39 : 54 : 10 respectively. Some *durum*-like plants with flat ears were distributed to the *turgidum*-group and some *lanceolatum* to the *vulgare*-group, as already mentioned. A control of F_3 and subsequent generations has verified the distribution of the different groups. The *contractum* plants sometimes had flattened ears and then strikingly resembled the »*compressum*» forms described by KAJANUS (1923) and belonging to the *vulgare* × *dicoccum* cross. Among the *vulgare*-like plants there are many speltoid heterozygotes, segregating in F_3 in approximately 3 *vulgare* : 1 *speltoides*. Actually

the total figures from 11 segregating F_3 families were 339 *vulgare* : 132 *speltoides* ($\chi^2 = 2{,}30^{00}$).

10. THE SPELTOID PROBLEM IN THE LIGHT OF RESULTS FROM OTHER CROSSES.

The resemblance between *speltoides* out of *vulgare* × *turgidum* and the speltoid mutants in *vulgare* is worth special attention. The occurrence of speltoids in common bread wheat was first described and analysed by NILSSON-EHLE in 1917 and has ever since been the object of a great number of investigations and theories (NILSSON-EHLE, 1917, 1920, 1921, 1927; LINDHARD, 1922, 1923; ÅKERMAN, 1920, 1923 b, 1927 b; KAJANUS, 1923; WINGE, 1924; NILSSON-LEISSNER, 1925; HUSKINS, 1928, 1933; UCHIKAWA, 1941; etc.). A critical survey of the literature recently published by UCHIKAWA (1941) gives a review of the cytology and segregation of A, B, and C speltoids. The A type speltoid with its segregation of 1 normal : 2 heterozygotes : 1 homozygote and its normal chromosome number ($2n = 42$) is of special interest in this connection on account of its similarities to the SVT. UCHIKAWA rejects the theory of WINGE (1924) and HUSKINS (1928) that the A speltoids represent the genome combination *AA* . *BB* . *BB*, obtained by the pairing of the non-homologous genomes *B* and *C*. The frequencies of trivalents and quadrivalents were not higher in the heterozygous and homozygous speltoids than in the normal plants. UCHIKAWA therefore

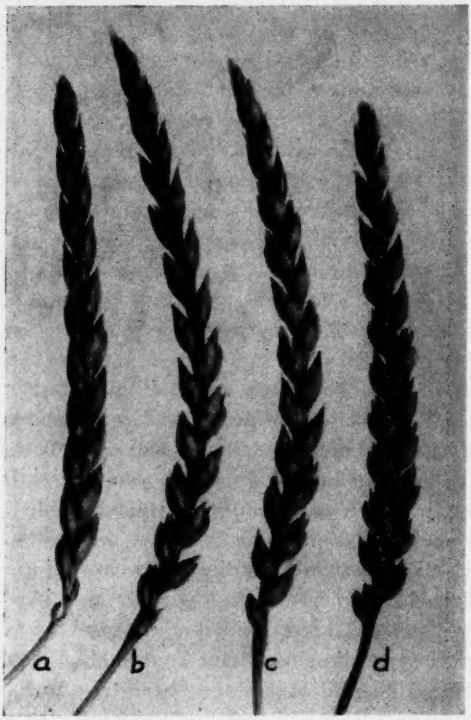

Fig. 6. *a* = Hohenheimer Spelz, *b*—*c* = *speltoides* lines of the 6000-series, *d* = Beardless Speltoid.

goes back to NILSSON-EHLE's original theory of a complex mutation and, moreover, points out its probable localization to the *C* genome, where the mutation of two inhibiting genes can bring the factors of speltoid glumes and beardness of the *B* genome into action.

In spite of the similarities between the two kinds of *Spelta*-like 42-chromosomal wheats (SVT and SV) and *Spelta* itself the interrelations of these wheats have not been made the subject of any exhaustive investigations. WATKINS (1940) has, however, discussed their places in his scheme of the *K* factors, concluding thus: »The gametic formula of *T. vulgare* is kKK^d. Speltoid mutants would then arise by an internal segregation that substitutes *K* for *k*, giving KKK^d, the formula already deduced for speltoid» (= SVT).

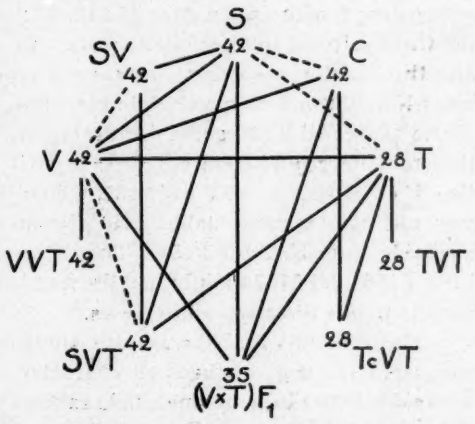

Fig. 7. Scheme of the cross combinations described in Chapter II: 10. (Dotted lines = crosses studied only by previous authors.)

In order to study these relationships the *speltoides* of *vulgare* × *turgidum* have been used by me for different cross combinations (Fig. 7), viz. SVT × S (crosses Nos. *30*, *31*, *43*, *45*, *48*, *50*, *52*, *53*), SVT × SV (crosses Nos. *63*, *64*, *75*, *76*), and SVT × T (crosses Nos. *44*, *46*). Some other combinations which are interesting in this connection have also been studied, viz. SV × S (cross No. *68*), S × V (crosses Nos. *2*, *3*). The backcrosses (V × T) F_1 × V (crosses Nos. *16*, *18*, *19*, *20*, *22*) and (V × T) F_1 × T (crosses Nos. *17*, *21*, *23*) and the combination (V × T) F_1 × S (cross No. *41*) are also reviewed here. Finally, the relations between *contractum* and *compactum* are affected in the combinations C × TcVT (crosses Nos. *29* and *59*), T × TcVT (cross No. *57*), (V × T) F_1 × C (cross No. *42*), and V × C (cross No. *83*).

(1) *SVT × S.* — The *speltoides* plants (SVT) have tough rachis and are usually more hairy at the rachis nodes than *Spelta*. In both these characters and also in the density of the head and the shape of the empty glume, in which characters the parents also show some differences, F_2 (comprising in all 3032 individuals) presented a com-

pletely continuous variation in all the crosses. Classification was consequently impossible. All the plants in F_2 and also in F_3 belonged to the *speltoides* or *Spelta* type.

In other characters, however, clear segregation was observed in F_2. Beardness thus gave normal 3 : 1 ratios in a number of crosses (No. *43*—349 beardless : 124 bearded; No. *48*—322 : 105; No. *50*—333 : 115; No. *52*—146 : 44; No. *53*—104 : 29) and also pubescence (No. *45*—683 pubescent : 222 glabrous). In one cross, viz. No. *31*, the segregation 156 beardless : 13 bearded was counted in F_2, indicating 15 :1 (χ^2 for 3 : 1 = 27,000***, χ^2 for 15 : 1 = $0{,}600^{000}$). In F_3, however, the three segregating families sown gave 28 : 12, 37 : 12, and 28 : 13. Consequently the three selected mother plants were only heterozygous in one factor, and the bifactorial segregation was not repeated among them. In the cross No. *30* some extremely late plants appeared in F_2 and also in F_3. These plants did not shoot heads before the last days of July and therefore no grains were developed. It is probable that the parental line of cross No. *30*, SVT 6163/167, which was crossed while still in F_4, was not cytologically stable. No chromosome counts are, however, available from SVT 6163/167. The corresponding F_5 lines, SVT 6163, 6165, 6166, and 6167 mainly had the numbers 2n = 40—42 and showed no late plants directly or in crosses.

The cross SVT × S was also studied by WATKINS (1940) with congruent result as to the spelt characters. He states that *Spelta* and *speltoides* both differ by one single factor from *vulgare*. The formulae *Spelta* = AB, *vulgare* = Ab, *speltoides* = ab would, however, presume the occurrence of a certain number of *vulgare* in F_2 of the cross SVT × S. These do not occur, and the formulae *Spelta* = $KK \,.\, K^sK^s \,.\, K^dK^d$, *vulgare* = $KK \,.\, kk \,.\, K^dK^d$, *speltoides* = $KK \,.\, KK \,.\, K^dK^d$ seem to completely cover the relations (Fig. 10: VII).

(2) *SVT × SV*. — The differences between *speltoides* from *vulgare* × *turgidum* and speltoid mutants of A type are not easily detectable. The rachis of SV is, however, not so hairy as that of SVT (see Fig. 6). Further, the heads are often extremely lax and prolonged in SVT. In the shape of the grain there are also some differences, the grains of SV being shorter and more *vulgare*-like, the grains of SVT on the other hand longer and more *Spelta*-like. In F_2 of the cross SVT × SV (comprising about 1500 individuals) all plants were speltoids, but the type varied continuously and intermediately between the parental types. The same picture was given in F_3. The SVT line 6177, which was used as parent in the cross No. *63*, was counted in 1936, when the cross was

carried out, and also in 1940 and showed good constancy, 2n = 42. The other SVT parental line 6178 was not counted, it is, however, a sister line of 6177 from the same F_2 plant, and has bred true to type just as 6177. The beardless and bearded SV forms which were used in these crosses were supplied by Prof. NILSSON-EHLE and belong to his A speltoids. They have been counted several times by me and show constantly 2n = 42.

I have not found any reports of the cross SVT × SV in the previous literature. The results obtained here confirm the genetical equalities of the two forms. It is true that differences in ear density, rachis

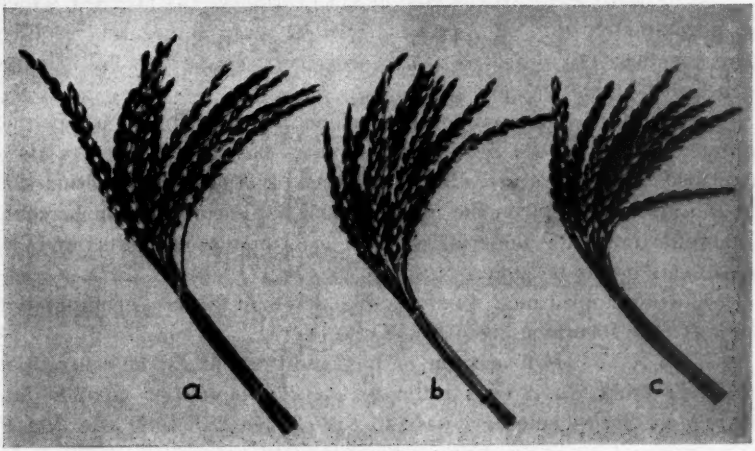

Fig. 8. Cross No. *63*, parents and F_2. *a* = *speltoides* (SVT 6177), *b* = F_2, *c* = Bearded Speltoid (SV).

hairiness, and grain shape can be observed between them, but factors outside the *K* complex may be responsible for that. Probably the ear density factors play some rôle.

(3) *SVT* × *T*. — This cross is pentaploid, and the segregation in F_2 is consequently complicated by sterility and other irregularities. However, cross No. *44* contained 51 *turgidum*-like plants, 172 intermediate *turgidum-Spelta*, 46 *speltoides*, and the corresponding figures of cross No. *46* were 46 : 91 : 11. No *vulgare*-like or *compactum*-like types with soft glumes were observed in F_2 or F_3 (Fig. 10: VIII). The parental lines of SVT in these two crosses were counted in 1936, the year of crossing, and showed SVT 6017: 2n = 41—42, SVT 6020: 2n = 40—42; in 1940 SVT 6017 was practically constant, 2n = 42.

WATKINS (1928) also reports that he has studied the cross SVT × T. In F_2 he found only *turgidum*, speltoids and intermediate types, with the exception of two plants, which were heterozygous speltoid-*vulgare* and probably had arisen from natural crossing. The variation in F_2 was in other respects continuous and was not analysed any closer.

(4) *SV* × *S*. — In cross No. *68* beardless speltoid mutant and bearded spelt were combined. F_2 comprised 518 individuals varying continuously within the bounds marked by the parental characters. It was impossible to separate different types. This was also the case in 10 F_3 families of the cross.

As to beardness F_2 segregated in 374 beardless : 144 bearded, which is approximately 3 : 1 ($\chi^2 = 2{,}1647^{00}$). In F_3 seven segregating families gave together 110 beardless : 47 bearded ($\chi^2 = 2{,}0403^{00}$). The surplus of bearded plants was not significant.

In spite of all discussions concerning the relationship of speltoid mutant and *Spelta* the results of this cross have not been previously described in the literature. NILSSON-LEISSNER (1925) has reported that he had an F_2 growing in the field in 1925, but according to a personal communication the material showed continuous variation and no classification was possible.

The non-appearance of *vulgare*-like plants in this cross combination supports the formulae given by WATKINS.

(5) *S* × *V*. — On account of the behaviour in F_3 the segregation of F_2 in cross No. *2* was estimated at 27 *vulgare* or *compactum* : 65 *vulgare-Spelta* heterozygotes : 26 *Spelta*. In the same way the F_2 segregation of cross No. *3* was estimated at 14 *vulgare* or *compactum* : 21 intermediate : 9 *Spelta*. Nine segregating F_3 families of cross No. *2* were also assorted, though only into two groups, giving in all 845 *vulgare* and intermediate : 325 typical *Spelta*.

This species combination has been thoroughly studied by several earlier investigators (cf. NILSSON-LEISSNER, 1925). The ratio 3 *Spelta* : 1 *vulgare* is stated in most cases, which agrees with WATKINS's formulae of the *K* complex (Fig. 10: XII).

(6) (*V* × *T*) F_1 × *V*. — The number of plants in these back-crosses was very small, viz. 19 in all the five crosses together. Only two of these plants proved to give constant *vulgare*-like F_2 without keel. Of the other 17 plants five varied in F_2 as to the keel development, but contained no *speltoides*. Finally, 12 F_2 families segregated in *vulgare* : *speltoides*.

WATKINS (1927 b), studying the same combination, found that nine

plants of altogether 18 gave *vulgare*, *speltoides* and intermediate forms in the next generation, three were constantly keelless, and the remaining six varied as to the keel and were difficult to classify. Presuming (V × T) F_1 to have the formula *KK . Kk . K*^{d} and *vulgare KK . kk . K*^{d} *K* the ratio 1 *Kk* : 1 *kk* is expected (Fig. 10: II). WATKINS interprets, however, the segregation found by him as being 15 *Kk* : 3 *kk*, and admits that the agreement is not good.

In my own material 12 plants are classed among *Kk*, but in the other seven plants I have found no signs of real *speltoides* characters, and consequently they are named *kk*. In the *Kk* families the total segregation was 465 *vulgare* and intermediate plants : 165 *speltoides*.

(7) (*V* × *T*) F_1 × *T*. — Also here the number of plants was small, and only 27 plants were obtained directly in all the three crosses, 15 of which gave only *turgidum*-like plants in the next generation, while the remaining 12 gave segregation in *turgidum* and keelless, dense *contractum*.

WATKINS (1927 b) reports 32 *KK* : 26 *Kk* obtained in this cross, expecting the ratio 1 : 1 (Fig. 10: III). My own figures of 15 *KK turgidum* : 12 segregating *Kk* are in good agreement with expectation. The *Kk* plants would give 25 % *kk contractum* in F_2 if no disturbances occur on account of the univalents from the *C* genome or for other reasons. It proved, however, impossible to count the segregation of keel development and ear density in these families.

(8) (*V* × *T*) F_1 × *S*. — Only one plant was obtained in this cross. The plant was speltlike, and gave rise to an F_2 family in which all plants were speltlike. Segregation was, however, counted in 77 pubescent : 32 glabrous.

If (V × T) F_1 has the formula *KK . Kk . K*^{d} and *Spelta KK . K*^{s}*K*^{s} . *K*^{d}*K*^{d}, the cross will give 1 *K*^{s}*K* : 1 *K*^{s}*k* (Fig. 10: IV). The latter type would give *int. al. kk vulgare* in the next generation. The single plant obtained from cross No. *41* was evidently of the former type, since no segregation in the type occurred in F_2.

(9) *C* × *TcVT*. — The parents are very alike in type, both having short straw, often curved just under the head, dense and short ears, glumes without keel, and short and plump grains. The combination, which is pentaploid, is consequently very interesting and has not been studied before.

In cross No. *29* all 32 F_2 plants were *compactum*-like but many of them were more or less sterile. In F_3 most plants were also *compactum*-like, but in three families of nine segregation in the density and length

of the ear appeared. Also in cross No. *59* all F_2 plants were completely uniform in type, all 75 — belonging to four families — being *compactum*-like. This F_2 is illustrated in Fig. 9, where the constancy of the head shape is very evident, but also the variation in plant height. In F_3 12 families were studied; ten of them with altogether 105 plants

Fig. 9. Cross No. *59*, parents and F_2. a = *contractum* (TcVT 6153), b—e = F_2, f = Beardless Club (C).

were unchanged *compactum*-like, but in two families laxer *vulgare*-like plants also occurred.

Compactum is not included in WATKINS's scheme. The results obtained in the cross C × TcVT suggest that the *A B* genomes may be alike as to the *K* factors and that the presence or absence of the *C* genome does not influence the type thus constituted. Consequently *compactum* may be described as $KK \,.\, kk \,.\, K^dK^d$, just as *vulgare* with regard to the *K* komplex (Fig. 10: IX). The occurrence of *vulgare*-like plants in

F_3 will be discussed later on (Chapter IV). The TcVT line 6153 of cross No. *59* was counted in 1936, when the cross was carried out, and had only 2n = 28. The constancy of this line seems to be very good. The other TcVT line, 6221, used in cross No. *29* as early as 1935, was also counted in 1936 and then showed 2n = 28. Like 6153, it has always been constant in type, but is considerably later in shooting and ripening.

(10) *T* × *TcVT*. — There is only one cross of this kind, No. *57*. F_1 resembled *turgidum* more than it did *contractum*, the heads being rather long and heavy. In F_2 segregation was observed as to the head type and keel development. Only 49 plants out of 404 were comparatively *contractum*-like, but the classification was very difficult to carry out. As already described, the segregation in beardness and pubescence was quite normal, 3 : 1 (Table 15). The TcVT line 6043 is a typical *contractum* with 2n = 28, counted in 1936 when the cross was carried out.

If *turgidum* is *KK* . *KK* and *contractum* *KK* . *kk* the expectation is 25 % *contractum* in F_2 (Fig. 10: X). The continuous variation made it impossible to test the agreement. This finding is really surprising, since the two types are easy to separate in F_2 of V × T, where they also appear together.

(11) (*V* × *T*) F_1 × *C*. — The cross No. *42* gave six F_1 plants, which were different in type, two being *vulgare*-like, three *compactum-vulgare*, and one exactly like the male parent *compactum*. In F_2 the *vulgare*-like plants segregated in *compactum* : *vulgare* : *speltoides* (16 : 8 : 1 and 15 : 49 : 3 respectively), the *compactum-vulgare* only in *compactum* : *vulgare* (40 : 11, 33 : 13, 34 : 26 respectively), and the *compactum*-like plant, finally, gave an offspring of 97 individuals, all bearded club.

If we make the assumption that *compactum* has the same *K* formula as *vulgare*, this cross combination would be expected to give the same quotient of 1 *Kk* : 1 *kk* in F_1 as the back-cross (V × T) F_1 × V (Fig. 10: V). The actual quotient seems to be 2 *Kk* : 4 *kk*. The *Kk* plants gave too few *compactum*, probably on account of the difficulties to distinguish lax *compactum-speltoides* and *vulgare*. As to the *kk* plants, segregation in *compactum* : *vulgare* is expected in all of them, and the homozygous *compactum* plant is consequently difficult to explain. The possibility that it represents an admixture of pure Bearded Club cannot be completely left out of account. In the segregating F_2 families with *kk* the agreement with 3 *compactum* : 1 *vulgare* is good in two cases but less good in the third one.

(12) *V* × *C*. — An F_2 of this cross (No. *83*) was assorted in the

Wheat Breeding Department at Svalöf in 1929 by Dr. GÖSTA ANDERSSON. The segregation was 1993 *compactum* and intermediate : 712 *vulgare*, which represents a rather good 3 : 1 ratio ($\chi^2 = 2{,}5199^{00}$).

IX.
C K K k k K4C K4C 42
TcVT K K k k 28
F1 K K k k K4C 35
C K K k k K4C K4C 35-42
TcVT K K k k K4C 28-35
X.
T K K K K 28
TcVT K K k k 28
F1 K K K k 28
T K K K K 28
TcVT K K k k 28
XI.
V K K k k K4C K4C 42
C K K k k K4C K4C 42
F1 K K k k K4C K4C 42
V K K k k K4C K4C 42
C K K k k K4C K4C 42
XII.
V K K k k K4 K4 42
S K K K4 K4 K4 42
F1 K K K4 k K4 K4 42
S K K K4 K4 K4 42
V K K k k K4 K4 42
XIII.
S K K K4 K4 K4 K4 42
C K K k k K4C K4C 42
F1 K K K4 k K4C K4C 42
C-S K K K4 K4 K4C K4C 42
S K K K4 K4 K4 K4 42
C K K k k K4C K4C 42
V K K k k K4C K4C 42
XIV.
S K K K4 K4 K4 K4 42
T K K K K 28
F1 K K K4 K4 K4 35
S K K K4 K4 K4 K4 35-42
SST K K K K4 K4 35-42
Dic. K K K4 K4 K4 28-35
T K K K K K4 28-35

Fig. 10. Schemes of the inheritance of the *K* complex. The formulae of the parents, F_1, and homozygous F_2 types. The corresponding chromosome numbers are given to the right of each formula. The different cross combinations are described in Chapter II: 10.

The same results have been reported by several previous authors (NILSSON-EHLE, 1911; KAJANUS, 1923; and others). According to NILSSON-EHLE's (1920) investigations on compactoid segregations in speltoids, a compactoid factor *C* is linked to the speltoid complex, but the linkage is markedly looser than between beardness and the speltoid

complex. These three factors would therefore be localized to the same chromosome. UCHIKAWA (1941) presumes that the compactoid factor belongs to the *C* genome, where inhibitors for the speltoid and the beardness factors would also be found. This presumption is in good agreement with the presumption of identical *K* formulae (*KK . kk . K^dK^d*) for *vulgare* and *compactum* but of differences in other parts of the *C* genome, provided the compactoid and the *compactum* factors are identical (Fig. 10: XI).

In addition to these cross combinations studied by me, I shall also shortly refer to some other crosses which are interesting in this connection, namely V × SV, V × SVT, S × C, and S × T.

(13) *V × SV* (NILSSON-EHLE, 1920, 1927). — Several different F_2 combinations gave concordantly 1 *vulgare* : 2 heterozygotes : 1 speltoid, when the A speltoid was involved. Linkage between beardness and the speltoid complex was established.

(14) *V × SVT* (KAJANUS, 1923; WATKINS, 1928). — In F_2 the segregation was 1 *vulgare* : 2 heterozygotes : 1 *speltoides*. Beardness and *speltoides* type proved to be linked (Fig. 10: VI).

(15) *S × C* (KAJANUS, 1923; NILSSON-LEISSNER, 1927). — In F_2 *Spelta*, *compactum* and *vulgare* occurred and also a great number of intermediate *compactum-Spelta* plants, which have been differently assorted by the investigators (Fig. 10: XIII). Both of them, however, presume a bifactorial segregation. KAJANUS observed linkage between beardness and the spelt complex, but the *compactum* gene seemed to be independent of this linkage.

(16) *S × T* (WATKINS, 1928). — The variation in F_2 of this pentaploid cross was continuous, but *turgidum*, *Spelta*, speltoids, and probably also *dicoccum* types were observed. The formula of the latter was presumed to be *KK . K^sK^s*, i. e. 28-chromosomal spelt (Fig. 10: XIV). The number of individuals with tough rachis seemed to be underrepresented.

The results of the different cross combinations reviewed above are also illustrated by the scheme in Fig. 10, where the *K* formulae of the parents, F_1, and the homozygous F_2 types are recorded. The formulae given by WATKINS for *vulgare*, *Spelta*, *turgidum*, *dicoccum*, speltoid mutant (SV, A-type), *speltoides* (SVT), and *contractum* (TcVT) are verified here. *Compactum* has been added, representing the same *K* formula as *vulgare* but differing from *vulgare* in the factor *C* of the *C* genome. (See also Chapter IV.)

III. STUDIES ON THE INHERITANCE OF WINTER-HARDINESS AND OTHER PHYSIOLOGICAL CHARACTERS.

1. POLYPLOIDY AND PHYSIOLOGY.

The ecology and especially the geographical distribution of related natural polyploids and diploids have been studied by many authors. According to MÜNTZING (1936) as well as FISCHER and SCHWANITZ (1936), the high-chromosomal races or species usually have a more northern or alpine distribution than the related diploids. Investigations on polyploidy frequency in different regions also point in the same direction (HAGERUP, 1932; TISCHLER, 1935; SOKOLOVSKAJA and STRELKOVA, 1938, 1940; FLOVIK, 1938; LÖVE and LÖVE, 1942, 1943; etc.). On the other hand, BOWDEN (1940) has found that subtropical or temperate representatives of otherwise tropical families have not as a rule higher chromosome numbers than the tropical members. Direct examples of polyploids less hardy than their diploid relatives are reported, for instance, within the species or genera *Vaccinium uliginosum*, *Oxycoccus* (HAGERUP, 1933, 1940); *Stellaria neglecta* (PETERSON, 1935; cf. MÜNTZING, 1936); *Campanula rotundifolia* (BÖCHER, 1936); *Vinca*, *Sequoia* (BOWDEN, 1940).

Several investigations have also shown that the natural polyploids usually possess a greater ability than the diploids to withstand other extreme life conditions. Examples of such are given by HAGERUP (1932, 1938) and ROHWEDER (1934). Reverse conditions are reported by GREGOR and SANSOME (1930). In the *Rumex Acetosella* group LÖVE (1940, 1942) has investigated diploid, tetraploid, hexaploid, and octoploid forms and found increase of cell size, osmotic pressure, leaf pigments, and p_H of sap, but decrease of oxalic and ascorbic acid contents with increased polyploidy.

As a general rule artificially induced polyploidy is combined with gigantism — increased cell size, bigger and especially broader and thicker leaves, thicker and often also longer stems, bigger flowers and fruits (cf. WETTSTEIN, 1924; DARLINGTON, 1937; etc.). In several cases exceptions from these rules have been found, which indicates that the genetical constitution of the mother plant has a great influence upon the result of chromosome doubling. The most striking examples of this influence on the morphology are given by PIRSCHLE (1942 a), who found remarkable differences in the effect of polyploidy inducement in different genotypes of *Impatiens balsamina*.

The physiological qualities of the artificial autopolyploids present

a very motley picture. Usually the bigger cotyledons give the polyploids a good start at the seedling stage, but the »diploids» soon surpass them in growth on account of more rapid cell divisions, and attain a higher production of fresh and dry matter. In time, however, there often comes »ein entwicklungsphysiologischer Umschlagspunkt» at which the polyploids catch up to the diploids and even outyield them, as SCHLÖSSER (1940) found in his *Lycopersicum* material. FABERGÉ (1936), who also worked with *Lycopersicum*, and HESSE (1938), working with *Petunia*, found that the autotetraploid reached just as high a fresh weight as the diploid but not higher. In *Nicotiana* (SMITH, 1939), *Solanum nodiflorum* (LARSEN, 1941), and *Linum* (LEVAN, 1942 a) this inversion point is not at all reached and the tetraploids are lighter and shorter at maturity than the diploids. In PIRSCHLE's (1942 a) *Impatiens balsamina*, finally, one form (»weinrot») showed superiority for the tetraploid, another form (»eosin») for the diploid, in the production of fresh and dry matter. Usually the artificial autopolyploids are later than the diploids and have a prolonged vegetation period, and sometimes this difference is so pronounced that in field trials the polyploids are found to be stunted in the autumn, a fact that must be remembered in this connection.

The resistance to cold and draught of the autopolyploids is but little investigated and most statements as to these qualities are founded upon observations on the dry matter content and the osmotic pressure of the cell sap. The dry matter content was usually lower in the artificial polyploid than in the corresponding »diploid» (HESSE, 1938; GREIS, 1940; PIRSCHLE, 1942 a, b; LEVAN, 1943; etc.). In the roots of sugar beets PETO and BOYES (1940) found, however, higher sugar content in triploids than in diploids, but LEVAN (1942 b) could not verify this difference in his material. SCHLÖSSER (1940), who also studied sugar beets, found that the diploid had higher dry matter content in the three first leaves, but the tetraploid higher in later appearing leaves. In contrast to this finding GYÖRFFY (1941 b) has observed in a great number of species that the dry matter content is initially about equal in tetraploids and diploids but that the latter subsequently show higher values. PIRSCHLE (1942 a) certainly found lower values in the autopolyploids of *Impatiens balsaminea* »eosin» and »rosa», but in the form »weinrot» the conditions were reversed. The relative contents of dry matter are consequently dependent upon the date of the investigation and the genetical composition of the diploid starting material.

A decrease of the osmotic pressure in autopolyploids was reported

by several authors (BECKER, 1931; SCHLÖSSER, 1936; HESSE, 1938; GREIS, 1940; etc.). GYÖRFFY (1941 a, b) found, however, that the relative osmotic pressures as between the ploids can be influenced by the stage of development and by external conditions. His experiments with different moisture and temperature in the climate chamber seem to indicate that the polyploids have a higher ability to adapt themselves to extreme environmental conditions.

In wild tomato and winter-rape SCHLÖSSER (1936) observed that the artificial tetraploids were more injured by cold than the diploids. KOSTOFF (1938), on the other hand, reports quite briefly that tetraploid tomato and *Nicotiana* were hardier than the corresponding diploids. Other brief notes concerning observations on the resistance to cold are also found in the literature. In *Avena* NISHIYAMA (1934) observed better winter-hardiness in polyploid species and in experimentally produced polyploids. MÜNTZING and PRAKKEN (1940) mention that triploid timothy-grass seems hardier than diploid, and RANDOLPH (1941) remarks that tetraploid maize has about the same resistance to draught, cold, and diseases as the diploid. In *Capsicum* the tetraploid seems to grow better in the greenhouse under winter conditions than the diploid, and several other autopolyploids studied by GYÖRFFY (1941 b) were more vigorous and turgescent in the dry climate chamber than the corresponding diploids. In PIRSCHLE's experiments in the climate chamber, on the other hand, the tetraploids of *Epilobium collinum* and *Stellaria media* (1941, 1942 c) seemed less resistant to draught than the diploids.

The artificial allopolyploids usually show the same tendency to gigantism in morphological respects as the autopolyploids (cf. DARLINGTON, 1937; SMITH, 1939; etc.). BECKER (1931) found that the osmotic pressure of allopolyploids in mosses was lower than in the diploids, but the decrease was not so pronounced as in the autopolyploids, and SCHWANITZ (1932) reported better assimilatory effect in allo- than in auto-polyploid mosses. PIRSCHLE (1942 b) found that allopolyploids of *Epilobium*, *Brassica*, and *Nicotiana* had a higher plant weight than the diploids but lower dry matter content, just as the autopolyploids.

The first impressions of »the polyploidy effect» in artificially produced material were in discrepancy with the conditions observed in nature. The polyploids in nature seemed hardier than the diploids, but the artificial polyploids at first mainly exhibited decreased dry matter content, osmotic pressure, and winter-hardiness. Later on these discrepancies have been reduced by fresh investigations. In view of

the greater variability of the artificial polyploids, established by KOSTOFF (1939), their greater power of adaptation, and the connection found between »polyploidy effect» and the genetic constitution of the starting material, the breach seems to be bridged over. If the doubling occurs in nature not one but a multitude of times, and the starting material possesses a definite range of variation, natural selection may have sufficient possibilities to choose polyploid forms viable in extreme climatical and ecological conditions of life.

In my own physiological investigations within the genus *Triticum* the comparison does not refer to artificial polyploids contra diploids, but to hexaploids and tetraploids extracted from the progeny of different crosses between *vulgare* (6x) and *turgidum* (4x). The aim has been to study whether any »polyploidy effect» could be observed as to cold-resistance and some related qualities, and whether such an effect, if any, ought to be ascribed to the polyploidy *per se* or to the gene combinations obtained.

2. THE NATURE OF WINTER-HARDINESS AND COLD-RESISTANCE.

For perennial or winter-annual wild or cultivated plants the ability of enduring the cold and other hardships of the winter often plays a decisive part for the existence of the plant. For the winter-wheats in our degrees of latitude, therefore, winter-hardiness is one of the most important varietal characters, and great attention has been devoted to this property in practical breeding work as well as in theoretical investigations (ÅKERMAN, 1923 a, 1924, 1927 a; etc.).

The overwintering of the wheat in practice is dependent upon a series of factors, for instance, the field factors, the climatic conditions, the sowing factors, and the choice of variety. The field factors include in their turn such factors as geographical position, neighbourhood, soil, and drainage. The climatic factors comprise temperature during different periods, the fall of rain and snow, the number and distribution of hours of sunshine, the winds, etc. The sowing factors include the development, germinating power and predisposition to fungous diseases of the seed, the time and method of sowing, etc. The varietal factor, finally, includes such characters as rhythm of development, resistance to low temperatures or persistent cold, resistance to draught, to frost actions in the soil, to suffocation by stagnant water, and to fungous diseases and insect attacks, etc. Consequently, the picture obtained in the experimental field at the end of the winter period is a product of complicated and interfering processes, which also give range to chance.

Artificial freezing trials can be carried out under conditions considerably better controlled. In these trials the proper cold-resistance of different varieties can be determined. Investigations of this kind conducted by ÅKERMAN and others show, however, that the agreement between the surviving ability in artificial trials and that in a series of field trials extending over several years is surprisingly good. This finding indicates that cold-resistance plays the most important part in the overwintering.

Many theories have been advanced to explain the nature of death from low temperatures. The most important phenomena seem to be the irreversible changes brought about in the plasm by the ice formed

TABLE 22. *Cold-resistance and sugar content in wheat varieties at Svalöf* (ÅKERMAN, *1927 a*).

Hardiness group in the field observations	Varieties ranked according to their cold-resistance in artificial freezing trials	Relative sugar content
I	Sammet	100
II	Svea II	87
IV	Thule II	67
»	Standard	66
»	Sol II	65
V	Pansar II	48
VI	Extra-Squarehead II	44
VII	Danish Smaahvede	41
VIII	Wilhelmina	39
IX	Perl summer-wheat	29
X	Halland summer-wheat	22

within the cells, and the dehydration and coagulation of the plasm colloids caused by the ice formed in the intercellular spaces (cf. ÅKERMAN, 1927 a; KESSLER, 1935; FUCHS, 1935). By the existence of certain protecting matters, e. g. sugar, which are dissolved in the cell sap, the temperature causing death will be lowered. At the advent of the winter-resting stage of wheat, when assimilation ceases on account of low temperature and lack of light, starch is transformed to sugar and the hardening begins. Usually there is a good parallelism in winter-stage wheat between dry matter content, sugar content, and osmotic pressure on one hand and the resistance to cold on the other. Exceptions are, however, found, which indicates that the resistance is also (VETUKHOVA, 1938) or initially (KESSLER, 1935) dependent upon the colloidal conditions of the plasm.

Several of the varieties used as parents in my crosses and as

standards in the freezing trials and other physiological experiments are already well-defined as to their cold-resistance and sugar content by ÅKERMAN's (1927 a) investigations. In Table 22, which is translated from his paper, a number of wheat varieties are ranked according to their field hardiness and cold-resistance in freezing trials. The table also gives the corresponding relative values of the sugar content (Sammet = 100). The parallelism between sugar content and resistance is very pronounced in this material.

3. MATERIAL AND METHODS EMPLOYED IN THE PHYSIOLOGICAL INVESTIGATIONS.

In order to obtain comparatively constant and euploid lines for the investigations on cold-resistance and other physiological qualities

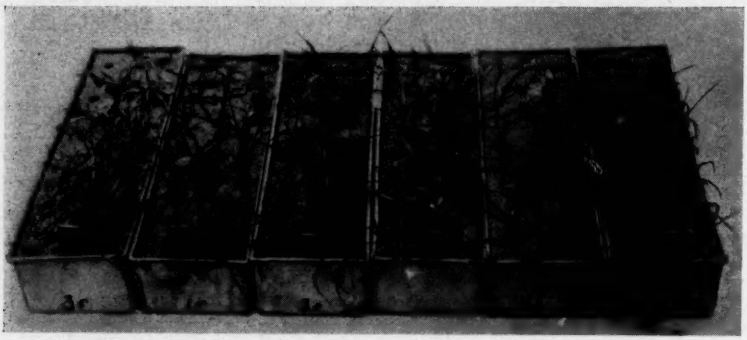

Fig. 11. Material from the freezing trial of 1935—36. 24 = VVT 6146, 22 = TVT 6139, 20 = Red Rivet, 19 = 0984 d, 23 = TVT 6140, 8 = SVT 6021. The resistance values are noted on the boxes.

the lines belonging to the 6000-, 8000-, 9000-, and 0000-series were selected in the manner described in Chapter II: 2. The origin, chromosome number, and morphological type are reported in Chapter II: 8 and in Tables 17—20.

1935—36. — The first artificial freezing trials were carried out in the winter 1935—36 with material from the 6000-series (F_5). From every line 90 grains were reserved for the freezing trials, 10 for the chromosome counts, and 90 (sometimes fewer) were sown in the field.

In the freezing trials of the first year 122 numbers were included, viz. 87 F_5 lines from the 6000-series (48—6x, 2—»5x», and 37—4x) and 35 standard varieties, *int. al.* the parental varieties 0984 d and Red Rivet each nine times for comparison.

The sowing was performed in wooden boxes filled with garden soil in cold beds. When the plants had obtained the three-leaf stage the material was transplanted in zinc boxes with sand (see Figs. 11—15). Each zinc box contained 30 plants, and consequently three boxes were

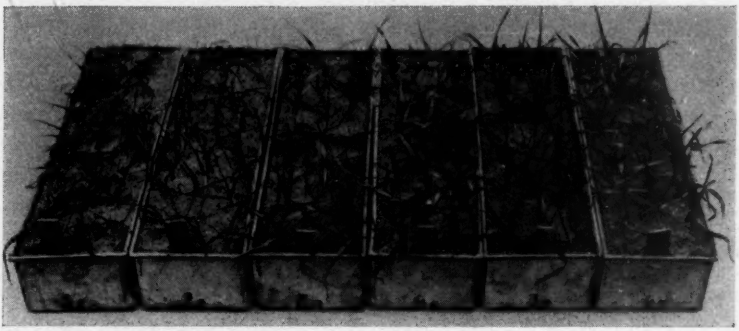

Fig. 12. Material from the freezing trial of 1935—36. 35 = Standard, 4 = VVT 6010, 20 = Red Rivet, 19 = 0984 d, 12 = VVT 6048, 36 = Sammet.

required of each line. These three boxes were frozen in different chambers and at different temperatures. The freezing trials were carried out in the Cold Laboratory of Sveriges Utsädesförening, and the same technique was used as in the yearly testing of the cold-resistance

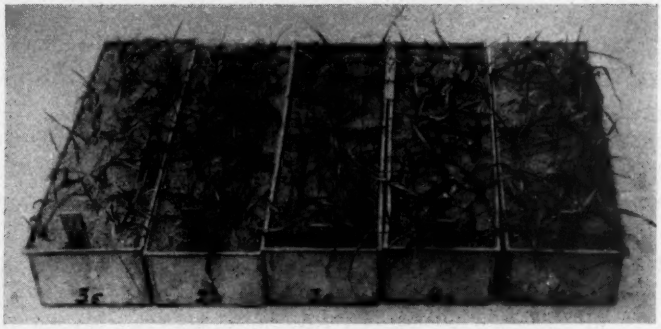

Fig. 13. Material from the freezing trial of 1935—36. 38 = Blue Spelt, 27 = SVT 6163, 20 = Red Rivet, 19 = 0984 d, 28 = SVT 6165.

of breeding material of winter-wheat and other overwintering cultivated plants (ÅKERMAN, ANDERSSON, and LINDBERG, 1935).

Immediately after the transplantation the material was placed in a greenhouse with a temperature of about + 2° C. After about three

days the boxes were placed in the antechamber of the freezing department for one day at — 2° C, and finally into the refrigerating chambers. This successive exposure of the plants to lower temperatures ensured their being hardened under uniform conditions before the

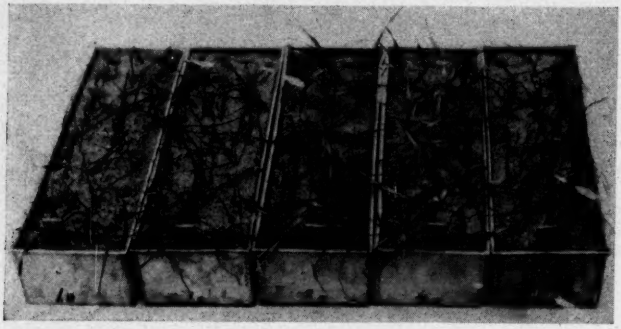

Fig. 14. Material from the freezing trial of 1935—36. 39 = Rivet Cambr. 4, 15 = TVT 6088, 20 = Red Rivet, 19 = 0984 d, 17 = TVT 6093.

freezing. At the freezing itself the temperature was also lowered slowly during about 24 hours down to the minima. These minima were chosen with due regard to the condition and composition of the material, and usually the three chambers were adjusted with an interval of

Fig. 15. Material from the freezing trial of 1935—36. 37 = Pubescent Club, 10 = TcVT 6041, 20 = Red Rivet, 19 = 0984 d, 11 = TcVT 6043.

about 2° C. After about 24 hours at the minima the temperatures were first slowly increased to about — 2° C in the chambers and the antechamber during about 24 hours, the doors of the chambers being opened, then to about + 2° C in the cold section of the greenhouse

until the soil had thawed after a couple of days. Finally the boxes were shifted to the warmer part of the greenhouse at about + 10° C.

The frost-damages were estimated after about a week according to a scale comprising five (1—5) degrees. Also half degrees were, however, estimated so that, for instance, 1,0 = completely dead plants, 1,5 = single plants with surviving green parts, 3,0 = plants about half-damaged, 5,0 = completely undamaged plants. The estimation was carried out in all my trials by three to four qualified persons quite independently. For each line and series of temperatures a figure was fixed upon from these estimations. The average from the three temperatures was finally calculated as the resistance figure of the line in question.

TABLE 23. *Temperatures in the freezing trials.*

Year	Trial	Nos. in the trial	Date of the start of freezing	Temperatures I	II	III
1935—36	A	1— 41	$^{11}/_{12}$ 1935	— 12,5°	— 14,5°	— 17,0°
»	B	42— 82	$^{17}/_{12}$ »	— 15,0°	— 15,5°	— 18,0°
»	C	83—122	$^{19}/_{12}$ »	— 15,0°	— 16,0°	— 18,0°
1936—37	A	1— 63a	$^{2}/_{12}$ 1936	— 13,0°	— 15,0°	— 17,0°
»	B	64—126	$^{5}/_{12}$ »	— 15,0°	— 17,0°	— 19,0°
1937—38	A	1— 63	$^{13}/_{12}$ 1937	— 14,0°	— 16,5°	— 19,0°
»	B	64—123	$^{16}/_{12}$ »	— 14,0°	— 16,5°	— 19,0°
1938—39	A	1— 63	$^{3}/_{2}$ 1939	— 13,0°	— 16,0°	— 18,0°
»	B	64—125	$^{7}/_{2}$ »	— 14,0°	— 16,0°	— 19,0°
1939—40	A	1— 63	Frozen in the open air			
»	B	64—126	» » » » »			

On account of the size of the material in the first year it was necessary to distribute the lines over three trials with 40—41 numbers in each of them. The temperatures employed in the three trials are seen in Table 23. It will be noticed that the temperatures in A were higher than in B and C, because the plants of the latter trials were expected to be better hardened.

In each trial the parental varieties 0984 d and Red Rivet occurred three times, once in each chamber. In trial A the resistance figure for 0984 d was 4,3, in B 3,3, in C 3,2, and the corresponding figures for Red Rivet were 3,4, 2,8, and 2,3. Starting from the averages 3,6 for 0984 d and 2,8 for Red Rivet a correction was made, all resistance figures in A being diminished by 0,6, all figures in B increased by 0,1, and in C by 0,5.

1936—37. — The experiment was repeated with mainly the same material as in the first winter. The sowing was carried out in a new

type of wooden boxes, each taking 7 × 30 plants. The freezing was then done in the same big wooden boxes without transplantation. In this way much space was saved in the chambers, and 140 numbers could be frozen in two trials. Of these numbers 76 belonged to the 6000-series (F_6; 44—6x, 2—»5x», and 30—4x) and 64 were standard varieties, 0984 d and Red Rivet being represented each eight times.

The temperatures are seen in Table 23. In trial A 0984 d had the resistance figure 4,3 and in B also 4,3, while Red Rivet had 3,4 and 3,1 respectively. At the correction the figures for A were diminished by 0,1 and the figures for B increased by 0,1.

1937—38. — This winter 123 numbers were frozen. Of these, 18 were 6000-lines (F_7; 10—6x, 8—4x) and 36 were standard varieties. The varieties 0984 d and Red Rivet were repeated nine times. The remaining 69 numbers belonged to the 8000-series. These lines were taken from F_4 of eight different crosses, viz. 28 from No. *5*, 7 from No. *6*, 8 from No. 7, 3 from No. *8*, 10 from No. *9*, 6 from No. *10*, and 7 from No. *11*. When all the lines were taken, 35—6x, 3—»5x», and 31—4x were included. The distribution of these three categories over the different crosses can be seen in Table 18 and Fig. 16. In five cases it was necessary to throw two related lines (habitually alike and derived from the same F_3 plant) into one in order to obtain sufficiently many grains for the freezing trial. Such combined numbers are named, for instance, 8005/6 in the freezing trial and correspond to Nos. 8005 and 8006 in the field trials of 1938.

The temperatures are given in Table 23. The resistance figures of 0984 d were 3,6 in trial A and 3,5 in B, and the figures of Red Rivet were 2,8 in A and 2,5 in B. A correction of — 0,1 in A and + 0,1 in B was consequently made.

1938—39. — The freezing trials comprised 125 numbers, viz. 16 from the 6000-series (F_8; 8—6x, 8—4x), 41 from the 8000-series (F_5; 19—6x, 1—»5x», 21—4x), 40 from the 9000-series (F_4), and 28 standard varieties (0984 d and Red Rivet nine of each). Of the lines from the 9000-series 28 were derived from different back-crosses (9000 A; 14—6x, 14—4x) and 12 from crosses between »typical» 6000-lines and different species (9000 B; 8—6x, 4—4x).

The freezing experiments were carried out at the temperatures recorded in Table 23. Since 0984 d had the resistance figures of 3,3 in A and 3,2 in B, and Red Rivet 1,4 and 1,7 respectively, no correction was made.

1939—40. — About the same number, 126, were sown in two groups

A and B. B contained 22 lines from the 8000-series (F_6; 11—6x, 11—4x), 27 from 9000 A (F_5; 14—6x, 13—4x), and 14 standard varieties. A contained 18 new lines (F_3) from the 0000 A-series (8—6x, 2—»5x», 8—4x), 28 from 0000 B (6—6x, 8—»5x», 14—4x), and 17 standards. Four of the standard varieties of trial A and B were common, viz. 0984 d, Red Rivet, Wilhelmina, and Sammet.

On account of the abundance of snow during the winter 1939—40 it was impossible to transfer the boxes to the freezing chambers at the intended time. The material therefore remained in the cold beds until April, when the winter-damages could be estimated. It proved then that no fungous diseases or other disturbing damages had occurred, and the estimates of cold-resistance seemed very successful, judging from the resistance figures of the standard varieties.

The chromosome counts on root tips have usually been performed on two to five plants of each line (sometimes up to ten). From each plant two to three unequivocal plates were counted. The technique of the Cyto-Genetic Laboratory at Svalöf has been employed. In all, about a thousand plants were counted.

In the field plots of the different years the same lines as occur in the freezing trials and a great number of sister lines from the same series were studied as to their germination, overwintering, constancy of type, date of shooting and ripening, plant height, resistance to diseases, etc.

Other physiological experiments. — A number of standard varieties of different species and some 6000- and 8000-lines were also made the subject of some investigations on dry matter content, sugar content, osmotic pressure, viscosity and permeability of plasm. These experiments were carried out in the years 1937—39 in the Plant-Physiological Laboratory at Svalöf.

4. FREEZING TRIALS IN THE YEARS 1936—1940.

1935—36. — The resistance figures of the standard varieties in the different years are recorded in Table 24. In order to give a survey of the relative hardiness I have also tried to rank the *vulgare* varieties in the hardiness groups established by ÅKERMAN (1927 a). The best result among the *vulgare* varieties was in 1935—36 obtained by the Central Swedish land-wheat Sammet, which was only slightly damaged in the leaf points. The *turgidum* varieties Red Rivet and especially Dinurum showed relatively good resistance, but otherwise the tetraploid standards were less hardy than the hexaploid varieties. The

TABLE 24. *The standard varieties in the freezing trials.*

Hardiness group	Variety	Resistance values in the trials of a 1935–36	b 1936–37	c 1937–38	d 1938–39	e 1939–40	Averages a—b 2 years	c—e 3 years	a—e 5 years
Vulgare:									
I	Sammet	4,2	$4,95^{2}$	$3,60^{2}$	$4,05^{2}$	$4,83^{3}$	4,58	4,16	4,33
II	Gluten	—	—	4,0	—	—	—	—	—
»	Minhardi	—	4,6	—	—	$4,65^{4}$	—	—	—
IV	0984 d	$3,59^{9}$	$4,28^{8}$	$3,59^{9}$	$3,24^{9}$	$4,73^{3}$	3,94	3,85	3,89
»	Ankar I	—	—	3,3	—	$4,45^{2}$	—	—	—
»	Standard	3,2	4,2	—	—	—	3,70	—	—
»	Sol III	—	—	3,2	—	$4,60^{2}$	—	—	—
V	0761	—	—	3,0	—	—	—	—	—
»	Pudel	—	—	3,0	3,0	—	—	—	—
»	01201	3,0	—	—	2,0	—	—	—	—
VI	Extra-Squarehead II	2,5	4,1	—	—	—	3,30	—	—
»	Skandia I	2,3	3,6	3,0	—	—	2,95	—	—
VIII	Wilhelmina	—	$2,59^{14}$	$2,35^{2}$	$1,35^{2}$	$2,50^{5}$	—	2,07	—
Compactum:									
—	Pubescent Club	3,7	4,7	—	—	—	4,20	—	—
—	Bearded Club	2,5	4,6	3,5	—	—	3,55	—	—
Spelta:									
—	White Spelt	3,5	—	3,5	3,3	—	—	—	—
—	Blue Spelt	3,3	4,2	—	—	—	3,75	—	—
Turgidum:									
—	Dinurum	3,2	3,4	3,2	2,7	$2,60^{3}$	3,30	2,83	3,02
—	Red Rivet	$2,80^{9}$	$3,24^{8}$	$2,63^{9}$	$1,58^{9}$	$2,03^{3}$	3,02	2,08	2,46
—	Rivet Cambr. 4	1,7	2,7	2,7	—	$1,90^{4}$	2,20	—	—
—	Compositum	—	—	$2,20^{2}$	1,3	$1,50^{2}$	—	1,67	—
—	Lusitanicum	1,7	1,1	—	—	—	1,40	—	—
—	Speciosum	1,3	1,1	—	—	—	1,20	—	—
Miscellaneous:									
—	Toivo Rye	—	5,0	—	—	—	—	—	—
—	Stål Rye	—	5,0	—	—	—	—	—	—
—	*Triticale* »C»	4,3	4,9	—	—	—	4,60	—	—
—	*Triticale* »A»	—	4,6	—	—	—	—	—	—
—	*aegilopoides*	—	—	3,5	—	—	—	—	—
—	*monococcum*	—	—	2,0	—	—	—	—	—
—	*durum hordeiforme*	1,5	1,1	—	—	—	1,30	—	—
—	*polonicum*	1,5	1,1	—	—	—	1,30	—	—
—	*Timopheevi*	1,5	—	—	—	—	—	—	—

The exponents indicate the number of individuals when more than one.

durum, *polonicum*, and *Timopheevi* varieties belonged to the spring forms. It was interesting to find that a form of *Triticale*, named »C» by MÜNTZING (1939), was just as hardy or even better than Sammet wheat. The parental varieties 0984 d *vulgare* and Red Rivet *turgidum* had an average resistance of $3{,}59 \pm 0{,}057$ and $2{,}80 \pm 0{,}060$ respectively; the difference is significant ($t = 9{,}634^{***}$).

The chromosome counts of the 6000-lines were not yet completed when the cold-damages were estimated in the freezing trials. It was, however, immediately evident that the *vulgare*- and *Spelta*-like lines were on an average more resistant than the *turgidum*-like lines. The correlation between type and hardiness was only broken in respect of the habitually *compactum*-like lines, which were not *compactum*-like in their hardiness but extremely susceptible to damage. The chromosome counts revealed that they were tetraploid and identical with KAJANUS's *contractum* form.

The hexaploid lines varied between 3,8 and 2,2 in resistance values, and the tetraploids between 3,2 and 1,5. The difference between the averages, $6x = 2{,}89$, $4x = 2{,}06$ (Table 25), is significant ($t = 8{,}74^{***}$). The two pentaploids were both very bad. It is interesting to note that the hexaploids on an average did not register the hardiness of the *vulgare* parent 0984 d ($-0{,}70 \pm 0{,}087$; $t = 8{,}05^{***}$), and that they were only a little better than Red Rivet ($+0{,}09 \pm 0{,}088$; $t = 1{,}02^{000}$). The tetraploids were clearly less hardy than the *turgidum* parent Red Rivet ($-0{,}74 \pm 0{,}090$, $t = 8{,}22^{***}$).

1936—37. — The number of standard varieties was even greater than the first year. Sammet was practically undamaged, and the American *vulgare* variety Minhardi and two *compactum* varieties were also given very high values at the estimation. The two rye varieties Toivo from Finland and Stål from Svalöf were completely undamaged. *Triticale* was this year represented by two types, named »C» and »A», both very hardy (the results of this test have been briefly mentioned by MÜNTZING, 1939). Dinurum was the best tetraploid variety, considerably better than the *vulgare* variety Wilhelmina from Holland, a fact that was also observed in the other experiments.

In all essential respects the trials of 1936—37 verified the results of 1935—36, and the following differences were found: 0984 — Red Rivet $= +1{,}04 \pm 0{,}162$, $t = 6{,}42^{***}$; $6x - 4x = +1{,}40 \pm 0{,}140$, $t = 10{,}00^{***}$; 0984 — $6x = +0{,}53 \pm 0{,}117$, $t = 4{,}53^{***}$; $6x$ — Red Rivet $= +0{,}51 \pm 0{,}143$, $t = 3{,}45^{**(*)}$; Red Rivet — $4x = +0{,}89 \pm 0{,}179$, $t = 4{,}97^{***}$. The extreme values in the hexaploid lines were 4,7—2,3, and in the tetraploids 3,7—1,3,

TABLE 25. *Results from the freezing trials.*

Year and material	Number of individuals	Resistance values; means and errors
1935—36:		
0984 d	9	3,59 ± 0,057
Red Rivet	9	2,80 ± 0,060
6000-ser.; 6x ..	48	2,89 ± 0,065
» 5x ..	2	1,80
» 4x ..	37	2,06 ± 0,067
1936—37:		
0984 d	8	4,28 ± 0,095
Red Rivet	8	3,24 ± 0,131
6000-ser.; 6x ..	44	3,75 ± 0,069
» 5x ..	2	1,65
» 4x ..	30	2,35 ± 0,122
Wilhelmina ..	14	2,59 ± 0,101
1937—38:		
0984 d	9	3,59 ± 0,070
Red Rivet	9	2,63 ± 0,126
6000-ser.; 6x ..	10	3,03 ± 0,100
» 4x ..	8	1,91 ± 0,129
8000-ser.;		
cross *5*; 6x ..	10	3,04 ± 0,145
» » 5x ..	1	2,3
» » 4x ..	17	2,46 ± 0,105
» *6*; 6x ..	5	2,84 ± 0,120
» » 4x ..	2	2,45
» *7*; 6x ..	4	3,35 ± 0,236
» » 5x ..	1	2,8
» » 4x ..	3	2,90 ± 0,153
» *8*; 6x ..	3	2,97 ± 0,147
» *9*; 6x ..	3	3,37 ± 0,168
» » 5x ..	1	2,5
» » 4x ..	6	3,40 ± 0,068
» *10*; 6x ..	5	3,14 ± 0,190
» » 4x ..	1	2,7
» *11*; 6x ..	5	2,56 ± 0,191
cross *11*; 4x ..	2	1,95
total; 6x	35	3,01 ± 0,073
» 5x	3	2,53 ± 0,147
» 4x	31	2,66 ± 0,097
1938—39:		
0984 d	9	3,24 ± 0,129
Red Rivet	9	1,58 ± 0,111
6000-ser.; 6x ..	8	2,19 ± 0,134
» 4x ..	8	1,94 ± 0,219
8000-ser.; 6x ..	19	2,28 ± 0,130
» 5x ..	1	2,3
» 4x ..	21	2,18 ± 0,110
9000 A-ser.; 6x	14	2,51 ± 0,115
» 4x	14	2,21 ± 0,122
9000 B-ser.; 6x	8	2,80 ± 0,235
» 4x	4	1,70 ± 0,071
1939—40:		
0984 d	3	4,73 ± 0,041
Red Rivet	3	2,03 ± 0,292
8000-ser.; 6x ..	11	3,85 ± 0,085
» 4x ..	11	1,65 ± 0,125
9000 A-ser.; 6x	14	4,03 ± 0,132
» 4x	13	1,99 ± 0,105
Wilhelmina ..	5	2,50 ± 0,164
Dinurum	3	2,60 ± 0,305
0000 A-ser.; 6x	8	2,45 ± 0,297
» 5x	2	2,55
» 4x	8	2,18 ± 0,232
Minhardi	4	4,65 ± 0,065
Rivet Cambr. 4	4	1,90 ± 0,145
0000 B-ser.; 6x	6	3,37 ± 0,211
» 5x	8	2,18 ± 0,195
» 4x	14	2,27 ± 0,116

and remarkable transgressions — positive and negative — consequently occurred.

1937—38. — Sammet had this year surprisingly low values, and Gluten was less damaged. These two varieties have only been compared

in my experiments this single year, but in other series of freezing trials carried out at Svalöf Sammet has proved better than Gluten. The difference between 0984 d and Red Rivet was significant ($+ 0{,}96 \pm 0{,}144$, $t = 6{,}67^{***}$). It is worth emphasizing that of the two diploid wheat species *aegilopoides* and *monococcum* which were represented, the former had very good hardiness (about the same as 0984 d). This is also well-known from the field observations in Svalöf, and presents a good example of the fact that hardiness is not confined to the hexaploid wheat species.

The 6000-lines gave the same results as earlier, and it will suffice to mention the difference between 6x and 4x lines, viz $+ 1{,}12 \pm 0{,}163$, $t = 6{,}87^{***}$.

In the 8000-series the number of lines from the separate crosses was not enough to enable any close comparisons between the crosses. The highest averages were, however, obtained in cross No. *9*, where the hardy *vulgare* variety Gluten was used as parent, while the lowest averages belonged to cross No. *11* with 0984 d as parent. The last-mentioned result was not expected, judging from the hardiness of the parents as illustrated by Fig. 16. Altogether the 6x lines of the 8000-series were significantly better than the 4x lines; $+ 0{,}35 \pm 0{,}122$, $t = 2{,}87^{**}$. The same tendency is seen more or less evidently within the different crosses, with the exception of cross No. *9*, where the 6x and the 4x lines were about equally hardy.

1938—39. — Only a few standard varieties were included. In the *vulgare* group Sammet was only slightly damaged, while Wilhelmina was nearly destroyed by the frost. In the *turgidum* group Dinurum was best as usual. The difference between 0984 d and Red Rivet was very pronounced ($+ 1{,}66 \pm 0{,}170$, $t = 9{,}76^{***}$).

As a general rule the difference between hexaploid and tetraploid lines was very small this year. In the 6000-series the difference was not significant ($+ 0{,}25 \pm 0{,}257$, $t = 0{,}97^{000}$) in spite of the big differences between the above-mentioned parents. (This result is partly due to a single, extremely high, value among the 4x lines, 3,3 for line 6194. The estimation of the damages is certainly true, but experimental errors of some other kind may have occurred.)

In the 8000-series the difference between 6x and 4x was also insignificant ($+ 0{,}10 \pm 0{,}171$, $t = 0{,}59^{000}$). The 4x group was still better than the best *turgidum* parent Red Rivet ($+ 0{,}60 \pm 0{,}156$, $t = 3{,}85^{***}$). Only one of the crosses, No. *5*, was fairly numerously represented; the

relations between the various groups did not deviate from the rest of the series.

The new series 9000 A and 9000 B were both small, and the different crosses included in them could not be compared. In 9000 A the difference between 6x and 4x was not significant ($+ 0{,}30 \pm 0{,}167$, $t = 1{,}80^{0}$). In 9000 B, however, the superiority of the hexaploids was quite definite ($+ 1{,}10 \pm 0{,}245$, $t = 4{,}49^{**(*)}$).

1939—40. — As already mentioned, the freezing was carried out in the open air. The winter was very hard and the differences in resistance between the extremes became manifest. Among the *vulgare* standards Sammet, Minhardi, 0984 d, and Sol III were only slightly damaged. The *turgidum* varieties, on the other hand, were severely damaged, and Dinurum was not significantly better than Wilhelmina ($+ 0{,}10 \pm 0{,}346$, $t = 0{,}29^{000}$). The superiority of 0984 d to Red Rivet was also now evident ($+ 2{,}70 \pm 0{,}295$, $t = 9{,}15^{***}$).

The differences in the different line series were very evident. Thus the hexaploid 8000-lines had on an average the resistance value 3,85 as against only 1,65 in the tetraploids ($+ 2{,}20 \pm 0{,}151$, $t = 14{,}57^{***}$). The extremes of the 6x lines were 4,2—3,4, of the 4x lines 2,3—1,1, i. e. no interference between the two distributions.

In the 9000 A-series the differences between the 6x and 4x averages were also significant ($+ 2{,}04 \pm 0{,}169$, $t = 12{,}07^{***}$).

Special interest attaches to the 0000 A-series, where the *turgidum* parent Dinurum had better cold-resistance than the *vulgare* parent Wilhelmina. To enable comparison the 0000 B-series, where the *vulgare* parent (Minhardi) was much better than the *turgidum* parent (Rivet Cambr. 4), was also included in the freezing trial. The four parent varieties in question had the resistance values Minhardi 4,65, Dinurum 2,60, Wilhelmina 2,50, and Rivet Cambr. 4 1,90. The difference between Dinurum and Wilhelmina was less than usual. The 0000-series belonged to F_4 and were consequently not so stable. Several lines habitually hexaploid or tetraploid proved pentaploid at the chromosome countings.

The 6x lines of 0000 A were a little hardier than the 4x lines, but the difference was not significant ($+ 0{,}27 \pm 0{,}377$, $t = 0{,}72^{000}$). Other comparisons within this group (6x, 4x and the parents), were also non-significant. In the 0000 B series, on the other hand, the 6x lines were evidently hardier than the 4x lines on an average ($+ 1{,}10 \pm 0{,}240$, $t = 4{,}58^{***}$). The pentaploids of this series gave very low figures; the difference 6x—5x is significant ($+ 1{,}19 \pm 0{,}287$, $t = 4{,}15^{**}$) but not the

difference 4x—5x ($+ 0{,}09 \pm 0{,}227$, $t = 0{,}40^{000}$). The hexaploids were not so good as Minhardi ($- 1{,}28 \pm 0{,}220$, $t = 5{,}82^{***}$) but better than Rivet Cambr. 4 ($+ 1{,}47 \pm 0{,}257$, $t = 5{,}72^{***}$). The tetraploids were also a little better than Rivet Cambr. 4, but this difference was not sufficiently significant ($+ 0{,}37 \pm 0{,}187$, $t = 1{,}98^{0}$).

5. WINTER-HARDINESS IN THE FIELD PLOTS.

Material belonging to the 6000-, 8000-, 9000-, and 0000-series, the parents and other standard varieties were sown in the field with the aid of marking boards. The grains were laid out in every hole of the boards with a distance of 5 cms in the row and 12,5 cms between the rows. The length of the board is 150 cms, and consequently 30 grains were sown in each row. Of each number, two or three rows were sown, corresponding to 60 or 90 grains in all. The number of plants was counted in the autumn before the beginning of the winter. In the spring the surviving plants were also counted when they were still small and easy to distinguish in the rows.

From the quotient, number of plants in the autumn: number of grains sown, the »germination percentage» was calculated, and from the quotient, number of surviving plants in the spring: number of plants in the autumn, the »overwintering percentage». A review follows of the observations made during the different years.

The winter 1935—36. — The average temperatures per month at Svalöf were: October + 8,3° C, November + 4,9° C, December + 1,1° C, January + 1,7° C, February — 2,5° C, March + 2,0° C. The lowest minimum temperatures observed were: October — 2,0° C, November — 2,5° C, December — 4,5° C, January — 8,0° C, February — 12,5° C, March — 4,0° C. The winter was relatively mild and only slight frost-damages occurred.

The plot-series 1936—366 not only included the 48 hexa-, 2 penta-, and 37 tetra-ploid 6000-lines of the freezing trial but also 52 other 6000-lines (F_5). The chromosome numbers of these last-mentioned lines were not counted, but on account of their appearance in 1936 and their behaviour in the subsequent generations they were separated into 43 hexa-, 1 penta-, and 8 tetra-ploids. At the statistical treatment of the figures the two groups »chromosome-counted» and »not chromosome-counted» lines were treated separately. It became evident, however, that the two groups could be thrown into one without objections, and the same experience was also made in the following years and in the other series. The correlation between chromosome number and

TABLE 26. *Germination in the field trials.*

Year and material	Number of individuals	Germination percentages; means and errors
1935—36:		
0984 d	9	60,2 ± 6,77
Red Rivet	9	71,3 ± 1,53
6000-ser.; 6x ..	91	59,8 ± 2,36
» 5x ..	3	44,0 ± 17,35
» 4x ..	45	52,2 ± 2,31
1936—37:		
0984 d	6	91,0 ± 2,34
Red Rivet	5	95,0 ± 3,35
6000-ser.; 6x ..	107	88,2 ± 0,96
» 5x ..	3	56,0 ± 14,57
» 4x ..	39	59,6 ± 3,83
1937—38:		
0984 d	3	94,0 ± 2,65
Red Rivet	3	88,3 ± 5,37
6000-ser.; 6x ..	38	82,3 ± 2,35
» 4x ..	21	58,2 ± 3,84
vulgare P	15	87,8 ± 3,79
turgidum P ..	11	89,0 ± 1,76
8000-ser.; 6x ..	45	85,8 ± 2,41
» 5x ..	4	63,0 ± 7,22
» 4x ..	54	72,0 ± 2,08
1938—39:		
0984 d	2	76,0
Red Rivet	2	87,5
6000-ser.; 6x ..	13	82,5 ± 2,20
» 4x ..	14	69,1 ± 3,19
vulgare P	9	90,4 ± 2,21
turgidum P ..	8	86,5 ± 2,65
8000-ser.; 6x ..	38	83,5 ± 1,56
» 5x ..	3	65,3 ± 11,78
» 4x ..	41	75,1 ± 2,50
vulgare S	6	96,0 ± 1,13
turgidum S ..	3	94,0 ± 3,79
9000 A-ser.; 6x	31	92,2 ± 0,99
9000 A-ser.; 5x	3	84,7 ± 3,94
» 4x	25	88,9 ± 1,68
hexaploid P ..	11	87,0 ± 2,41
tetraploid P ..	1	88
9000 B-ser.; 6x	42	82,7 ± 1,78
» 5x	5	78,4 ± 5,26
» 4x	13	82,4 ± 2,17
1939—40:		
0984 d	2	90,0
Red Rivet	2	91,5
6000-ser.; 6x ..	8	80,6 ± 4,45
» 4x ..	11	69,5 ± 3,13
vulgare P	9	88,4 ± 2,38
turgidum P ..	7	89,1 ± 1,67
8000-ser.; 6x ..	34	86,9 ± 1,29
» 4x ..	33	74,6 ± 3,39
vulgare S	5	93,8 ± 2,96
turgidum S ..	3	88,3 ± 3,54
9000 A-ser.; 6x	14	85,5 ± 3,48
» 4x	13	89,3 ± 1,92
hexaploid P ..	10	87,9 ± 2,51
tetraploid P ..	2	67,0
9000 B-ser.; 6x	9	87,6 ± 1,69
» 4x	4	82,3 ± 5,25
Wilhelmina ..	2	92,5
Dinurum	3	94,3 ± 3,19
0000 A-ser.; 6x	16	79,4 ± 2,86
» 5x	2	57,5
» 4x	9	81,1 ± 2,59
Minhardi	2	77,5
Rivet Cambr. 4	2	94,5
0000 B-ser.; 6x	6	69,8 ± 9,19
» 5x	8	67,0 ± 2,31
» 4x	15	84,0 ± 2,14
White Spelt ..	1	78
BeardlessSpelt-oid	1	85
0000 C-ser.; 6x	10	89,1 ± 1,66

P = parents, S = standard varieties.

type is, as already mentioned, very pronounced. In all, the observations of the winter 1935—36 consequently comprise 91 hexa-, 3 penta-, and 45 tetra-ploid lines. The parental varieties 0984 d and Red Rivet were represented by nine plots of each.

The germination in the autumn of 1935 was not satisfactory on account of the heavy rains in October. As seen in Table 26, the germination percentage was only 60,2 for 0984 d and 71,3 for Red Rivet. The hexaploids of the 6000-series germinated better than the tetraploids, the difference being relatively significant ($+ 7{,}6 \pm 3{,}30$, $t = 2{,}30$*). The pentaploids had a very poor germination.

The winter-hardiness was not severely tried, as shown in Table 27. The parental variety 0984 d was a little better than Red Rivet

TABLE 27. *Overwintering in the field trials.*

Year and material	Number of individuals	Overwintering percentages; means and errors
1935—36:		
0984 d	9	96,1 ± 2,74
Red Rivet	9	92,9 ± 2,11
6000-ser.; 6x ..	91	94,3 ± 3,09
» 5x ..	3	79,7 ± 13,39
» 4x ..	45	88,8 ± 2,23
1936—37:		
0984 d	6	95,0 ± 0,77
Red Rivet	5	79,4 ± 4,79
6000-ser.; 6x ..	107	84,3 ± 0,93
» 5x ..	3	50,7 ± 23,04
» 4x ..	39	63,3 ± 2,54
1939—40:		
0984 d	2	67,0
Red Rivet	2	6,0
6000-ser.; 6x ..	8	34,3 ± 8,31
» 4x ..	11	5,6 ± 1,72
vulgare P	9	55,4 ± 5,41
turgidum P ..	7	5,4 ± 2,65
8000-ser.; 6x ..	34	30,1 ± 3,16
8000-ser.; 4x ..	33	7,0 ± 1,44
vulgare S	5	51,0 ± 3,52
turgidum S ..	3	1,3 ± 1,41
9000 A-ser.; 6x	14	36,9 ± 3,05
» 4x	13	2,5 ± 1,05
hexaploid P ..	10	36,1 ± 6,40
tetraploid P ..	2	1,5
9000 B-ser.; 6x	9	35,3 ± 8,40
» 4x	4	11,8 ± 7,12
Wilhelmina ..	2	6,5
Dinurum	3	8,0 ± 3,79
0000 A-ser.; 6x	16	9,6 ± 2,69
» 5x	2	17,0
» 4x	9	3,1 ± 2,41
Minhardi	2	68,0
Rivet Cambr. 4	2	27,5
0000 B-ser.; 6x	6	31,5 ± 11,82
» 5x	8	8,3 ± 3,88
» 4x	15	15,2 ± 3,29
White Spelt ..	1	66
BeardlessSpeltoid	1	90
0000 C-ser.; 6x	10	74,4 ± 3,44

P = parents, S = standard varieties.

(+ 3,2 ± 3,46, $t = 0{,}92^{000}$), and the hexaploid lines a little better than the tetraploid ones (+ 5,5 ± 3,81, $t = 1{,}44^{00}$). The differences were not significant. The pentaploids had the lowest average values of overwintering.

The winter 1936—37. — Average temperatures: Oct. + 5,8° C. Nov. + 4,6° C, Dec. + 2,7° C, Jan. — 1,7° C, Febr. — 0,7° C, March + 0,1° C. Lowest min. temperatures: Oct. — 3,0° C, Nov. — 2,5° C, Dec. — 4,0° C, Jan. — 11,5° C, Febr. — 11,0° C, March — 12,0° C. The autumn was dry and the winter relatively mild.

In the plot-series 1937—259 firstly the 44 hexa-, 2 penta-, and 33 tetra-ploid 6000-lines of the freezing trials, secondly 63 other hexa-, 1 penta-, and 6 tetra-ploid lines of the 6000-series were included, all F_6. The parents 0984 d and Red Rivet were repeated six and five times respectively.

The germination was good for the parental varieties and for the hexaploid lines, poor for the tetraploid and pentaploid lines. The difference between the averages of 6x and 4x was very big and proved significant (+ 28,6 ± 3,96, $t = 7{,}22^{***}$).

The differences in winter-hardiness were a little more evident than in the preceding winter. Only 0984 d was practically undamaged (95,0 %). The difference between 0984 d and Red Rivet was established (+ 15,6 ± 4,85, $t = 3{,}22^{*(*)}$), and also the difference between 0984 d and the hexaploid 6000-lines (+ 10,7 ± 1,25, $t = 8{,}56^{***}$). Red Rivet was better than the tetraploid lines (+ 16,1 ± 5,42, $t = 2{,}97^{**}$). Of the hexaploids 84,3 % survived, of the tetraploids only 63,3 %, and this difference is also significant (+ 21,0 ± 2,72, $t = 7{,}72^{***}$). The pentaploids were very variable.

The winter 1937—38. — Average temperatures: Oct. + 9,1° C, Nov. + 2,6° C, Dec. — 1,4° C, Jan. + 0,9° C, Febr. + 0,8° C, March + 5,6° C. Lowest min. temperatures: Oct. + 0,5° C, Nov. — 9,0° C, Dec. — 13,0° C, Jan. — 10,0° C, Febr. — 8,0° C, March — 4,5° C. The autumn was dry and the winter very mild.

The plot-series 1938—246 contained 38 hexa- and 21 tetra-ploid 6000-lines (F_7). All of them, with the exception of 5 hexaploids, belonged to the chromosome-counted lines.

In the plot-series 1938—247 the following 8000-lines (F_4) were included: 45 (36) hexa-, 4 (3) penta-, and 54 (36) tetra-ploids; the figures in brackets representing the proportion with controlled chromosome numbers. The parental varieties were five Sol III, two Ankar II, two

Pudel, two Gluten, two 0761, and two 0984 d, i. e. 15 *vulgare*; four Compositum, and seven Red Rivet, i. e. 11 *turgidum*.

In the 6000-series all numbers germinated well with the exception of most tetraploid lines. The differences between 0984 d and 6x ($+ 11{,}7 \pm 3{,}54$, $t = 3{,}31^{**}$), between Red Rivet and 4x ($+ 30{,}1 \pm 6{,}60$, $t = 4{,}56^{***}$), and between 6x and 4x ($+ 24{,}1 \pm 4{,}50$, $t = 5{,}36^{***}$), are all significant.

In the 8000-series the pentaploid plants had the lowest average of germination. The differences between the *turgidum* parents and 4x ($+ 17{,}0 \pm 2{,}73$, $t = 6{,}23^{***}$), and between 6x and 4x ($+ 13{,}8 \pm 3{,}18$, $t = 4{,}33^{***}$), are significant, the difference between the *vulgare* parents and 6x is not significant ($+ 2{,}0 \pm 4{,}49$, $t = 0{,}45^{000}$).

The overwintering was throughout satisfactory and the small differences between the single plots could not be ascribed to differences in hardiness. No estimation of the overwintering percentage was consequently performed.

The winter 1938—39. — Average temperatures: Oct. + 9,4° C, Nov. + 7,2° C, Dec. — 0,2° C, Jan. + 0,6° C, Febr. + 2,4° C, March + 0,9° C. Lowest min. temperatures: Oct. ± 0,0° C, Nov. + 1,0° C, Dec. — 11,5° C, Jan. — 16,0° C, Febr. — 4,5° C, March — 7,0° C. The weather conditions in the autumn and winter were favourable.

The plot-series 1939—244 contained 13 hexa- and 14 tetra-ploid 6000-lines (F_8). In one of the hexaploids the chromosome number was not controlled.

The plot-series 1939—243 contained 38 (35) hexa-, 3 (3) penta-, and 41 (34) tetra-ploid 8000-lines (F_5). Nine plots of *vulgare* parents, viz. four Sol III and one of each Ankar I, Pudel, Gluten, 0761, and 0984 d were included, and also eight *turgidum*, viz. two Compositum and six Red Rivet.

To the plot-series 1939—242 belonged F_4 of 9000 A and 9000 B with standard varieties. 9000 A was represented by 31 (14) hexa-, 3 (0) penta-, 25 (13) tetra-ploids together with six *vulgare* varieties (two Sol III, two 0984 d, one Gluten, and one Pudel) and three *turgidum* (two Red Rivet, one Compositum). The plots of 9000 B comprised 42 (6) hexa-, 5 (0) penta-, and 13 (4) tetra-ploid lines. Eleven hexaploid parents (01201, Carsten V, Sammet, Extra-Squarehead II, White Spelt, Blue Spelt, VVT 6009/11, VVT 6051/64, VVT 6240/244, SVT 6163/167, and SVT 6179/185) and one tetraploid parent (TcVT 6221) were also represented.

The germination was good in most cases and only the following

differences proved significant: In the 6000-series the 6x were better than the 4x lines ($+ 13{,}4 \pm 3{,}88$, $t = 3{,}45$**). In the 8000-series the *vulgare* parents were better than the 6x lines ($+ 6{,}9 \pm 2{,}70$, $t = 2{,}56$*), the *turgidum* parents better than the 4x lines ($+ 11{,}4 \pm 3{,}64$, $t = 3{,}13$**), and, finally, 6x better than 4x ($8{,}4 \pm 2{,}95$, $t = 2{,}85$**). In the 9000-series (A and B) no differences are significant.

The winter 1939—40. — Average temperatures: Oct. $+ 5{,}0°$ C, Nov. $+ 3{,}9°$ C, Dec. $- 0{,}9°$ C, Jan. $- 5{,}6°$ C, Febr. $- 8{,}5°$ C, March $- 2{,}0°$ C. Lowest min. temperatures: Oct. $- 3{,}0°$ C, Nov. $- 9{,}0°$ C, Dec. $- 15{,}5°$ C, Jan. $- 21{,}0°$ C, Febr. $- 23{,}0°$ C, March $- 15{,}5°$ C. The autumn of 1939 was dry. The winter was exceptionally hard and long. In the spring of 1940 the weather was also very unfavourable, with drought and hard winds, and many sensitive varieties and lines had completely disappeared by the time of plant-counting.

The following material was studied. The plot-series 1940—505: 8 (8) hexa-, 11 (11) tetra-ploid 6000-lines (F_9). The plot-series 1940—504: 34 (33) hexa-, 33 (33) tetra-ploid 8000-lines (F_6), 9 *vulgare* (four Sol III, one of each Ankar I, Pudel, Gluten, 0761, and 0984 d), 7 *turgidum* (two Compositum, five Red Rivet). The plot-series 1940—503: 14 (13) hexa-, 13 (12) tetra-ploid 9000 A-lines (F_5) with 5 *vulgare* (two Sol III, one of each Gluten, Pudel, 0984 d) and 3 *turgidum* (one Compositum, two Red Rivet); 9 (6) hexa-, 4 (4) tetra-ploid 9000 B-lines (F_5) with 10 hexaploid parents (01201, Carsten V, Sammet, Extra-Squarehead II, White Spelt, VVT 6009/11, VVT 6051/64, VVT 6240/244, SVT 6163/167, SVT 6179/185) and 2 tetraploid parents (TcVT 6221). The plot-series 1940—501: 16 (8) hexa-, 2 (2) penta-, 9 (8) tetra-ploid 0000 A-lines (F_3 of cross No. *66*) with parents (Wilhelmina, Dinurum); 6 (6) hexa-, 8 (8) penta-, 15 (14) tetra-ploid 0000 B-lines (F_3 of cross No. *67*) with parents (Minhardi, Rivet Cambr. 4); 10 (0) hexaploid 0000 C-lines (F_3 of cross No. *68*) with parents (White Spelt, Beardless Speltoid).

The germination was relatively good. In the 6000-series the difference between 6x and 4x was not sufficiently significant but nearly so ($+ 11{,}1 \pm 5{,}44$, $t = 2{,}04^{0(*)}$). In the 8000-series the differences between *turgidum* and 4x ($+ 14{,}5 \pm 3{,}78$, $t = 3{,}84$***) and between 6x and 4x ($+ 12{,}3 \pm 3{,}63$, $t = 3{,}39^{**(*)}$) were established. In 9000 A and 9000 B no differences of germination were significant; in 9000 A the tetraploids have a very good average value. In 0000 A the difference between Dinurum and 4x was ascertained ($+ 13{,}2 \pm 4{,}11$, $t = 3{,}21$**). In 0000 B the hexaploids were inferior to the tetraploids but the difference was

doubtful ($-14{,}2 \pm 9{,}44$, $t = 1{,}50^{00}$). The pentaploids of this series were, on the other hand, clearly inferior to the tetraploids ($-17{,}0 \pm 3{,}15$, $t = 5{,}40^{***}$).

The percentages of overwintering plants are very interesting. As seen in Table 27, the tetraploid lines were usually severely damaged, and in reality this winter made an end of the investigations on the *vulgare* × *turgidum* hybrids.

At the comparison of the different means the significance was established in the following cases. The 6000-series: 6x—4x ($+28{,}7 \pm 8{,}49$, $t = 3{,}38^{**}$). The 8000-series: *vulgare* parents—*turgidum* parents ($+50{,}0 \pm 6{,}02$, $t = 8{,}31^{***}$); *vulgare*—6x ($+25{,}3 \pm 6{,}27$, $t = 4{,}04^{***}$); 6x—4x ($+23{,}1 \pm 3{,}47$, $t = 6{,}66^{***}$); 6x—*turgidum* ($+24{,}7 \pm 4{,}12$, $t = 6{,}00^{***}$). The 9000 A-series: *vulgare* standards—*turgidum* standards ($+49{,}7 \pm 3{,}79$, $t = 13{,}11^{***}$); *vulgare*—6x ($+14{,}1 \pm 4{,}66$, $t = 3{,}03^{**}$); 6x—4x ($+34{,}4 \pm 3{,}23$, $t = 10{,}65^{***}$); 6x—*turgidum* ($+35{,}6 \pm 3{,}36$, $t = 10{,}60^{***}$). On the other hand, the following differences were not sufficiently significant. The 9000 B-series: 6x—4x ($+23{,}5 \pm 11{,}01$, $t = 2{,}14^{0(*)}$). The 0000 A-series: 6x—4x ($+6{,}5 \pm 3{,}61$, $t = 1{,}80^{0}$). The 0000 B-series: 6x—4x ($+16{,}3 \pm 12{,}27$, $t = 1{,}33^{00}$); 6x—5x ($+23{,}2 \pm 12{,}44$, $t = 1{,}86^{0}$). The best overwintering was obtained in the 0000 C-series, where *Spelta* was crossed with speltoid.

6. CHROMOSOME NUMBER AND COLD-RESISTANCE IN THE DIFFERENT SERIES OF MATERIAL.

The 6000-series. — All lines of this series are derived from the cross No. *1*, 0984 d × Red Rivet. The cold-resistance of 0984 d is distinctly better than that of the other parental variety. This difference was statistically proved in the freezing trials of all the five winters. The hexaploid lines of the series were on an average better than the tetraploid lines. Only in the winter 1938—39 was significance not obtained in respect of this difference. As seen in Fig. 16, the average value of the hexaploid lines did not any year reach 0984 d. Some lines have given values higher than those of 0984 d but only in single years, and if we consider the averages of two to four years all lines were inferior to 0984 d. The tetraploid lines behaved in a corresponding way, the averages were lower than those of Red Rivet in three years of four (1938—39 furnishing the exception), and no single line has been awarded the hardiness of Red Rivet. The two pentaploid lines, which were tested in two years, were both very sensitive to cold.

The plant-counts in the field revealed that the germination of the

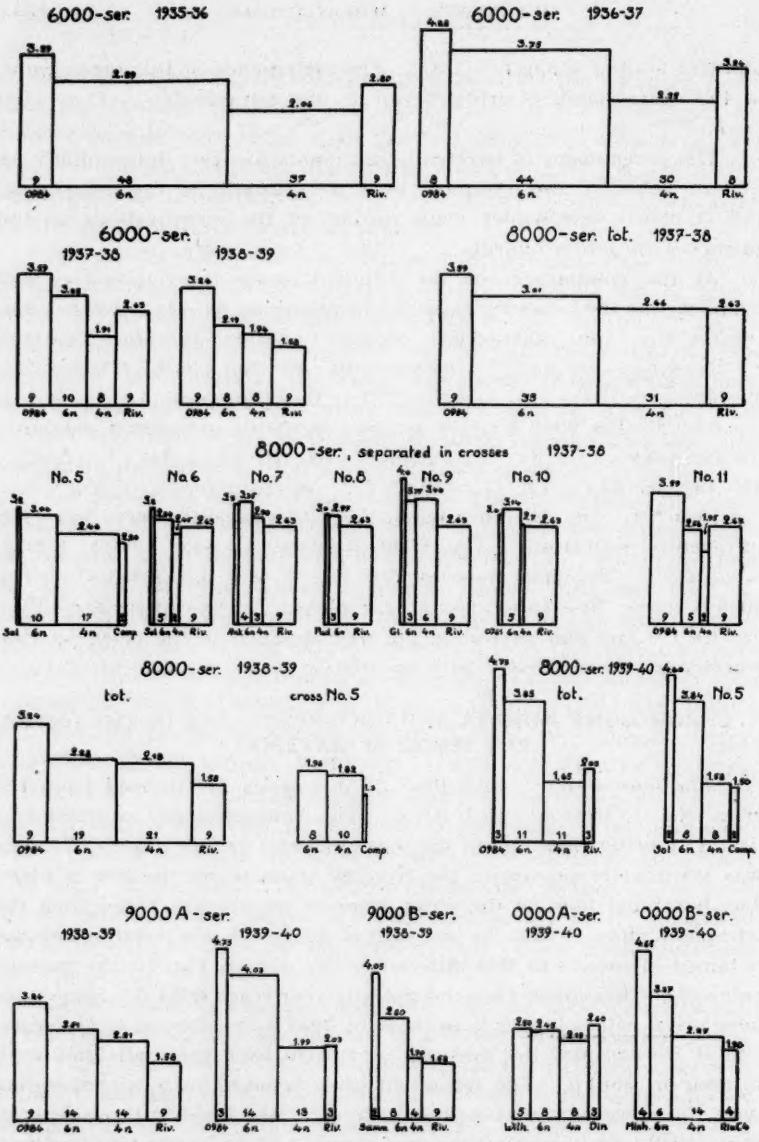

Fig. 16. Averages from the freezing trials of different years and series. The 6x and 4x derivatives (here named »6n« and »4n«) are placed between the parents or other standard varieties. The average resistance values are seen at the top of each pile, at the bottom the numbers of individual observations are noted. See text.

tetraploid lines was imperfect. Significant differences between 6x and 4x were obtained for this quality for all years in which the 6000-series was counted with the exception of 1939—40, when the difference was less reliable. The pentaploid lines also gave poor germination, but the hexaploids behaved quite normally.

The field overwintering was in full agreement with the results from the freezing experiments. In the hard winter 1939—40 the tetraploid lines were severely damaged.

The 8000-series. — In order to obtain a more comprehensive picture of the cold-resistance conditions in the progenies of *vulgare* × *turgidum*, the 8000-series were selected from seven different variety combinations. As seen in Fig. 16 and Table 25, the freezing experiments of 1937—38 comprised several lines from each cross. The *vulgare* parent was in every cross hardier than the *turgidum* parent. Cross No. *11* (0984 d × Red Rivet) showed big differences between 6x and 4x, just as did the identical cross No. *1* (the 6000-series). In the other crosses this difference was less pronounced. The best resistance was obtained in cross No. *9* (Gluten × Red Rivet), where the best *vulgare* parent was used. If all the 6x and the 4x lines of the 8000-series are compared, it will be found that the difference between the averages was relatively small in the year 1937—38 and very small in 1938—39. In 1939—40, however, a remarkably great difference was obtained. (It must be remembered, however, that the material was frozen in the open air that year.)

The germination in the field was less good in the tetraploid and pentaploid lines than in the hexaploid lines and the parental varieties.

The overwintering in the field in 1939—40 gave results agreeing with those from the »freezing trial». The *vulgare* parents were less damaged than the hexaploid lines; the *turgidum* parents and the tetraploid lines had largely disappeared.

The 9000 A-series. — The material was derived from different back-crosses. The 6x lines had higher cold-resistance values than the 4x lines both years, but the difference was significant only in 1939—40.

In the field trials no differences were established as to germination, but the winter-damages were much more serious in the tetraploid lines than in the hexaploid ones.

The 9000 B-series. — This series, which was derived from crosses between 6000-lines and different species, was tested only one year in the freezing experiments, viz. 1938—39. The 6x lines were significantly better than the 4x lines.

No real differences in germination were observed in the field. The counts of surviving plants in the spring gave the same picture as did the freezing trial.

The 0000 A-series. — At the testing of different standard varieties Dinurum had proved the hardiest *turgidum* variety available in my material. The variety was crossed with the sensitive *vulgare* variety Wilhelmina. As seen in Table 24, Dinurum had better resistance values than Wilhelmina in all the four years in which the two varieties were compared. The difference was least distinctive in 1939—40, when the 0000 A-lines from the cross were tested. The hexaploid lines were also better in this cross than the tetraploid lines, but the difference was not significant.

In the field trial only the pentaploids showed poor germination. The overwintering was very bad for both parents and also for the different lines, the hexaploids, however, showing a little higher mean percentage. The two pentaploid lines proved astonishingly hardy in the field as well as in the freezing trial.

The 0000 B-series. — The parental varieties of this series were especially different, Minhardi belonging to the hardiest *vulgare* wheats, while Rivet Cambr. 4 was less hardy than Red Rivet. As seen in Fig. 16 and Table 25, the progenies of the cross were intermediate between the parents at the freezing trial in 1939—40. The difference between 6x and 4x was significant. The pentaploid lines of this cross, which were relatively frequent, had lower resistance than the tetraploids.

The germination of this series gave a picture that differed from the usual one, the tetraploids showing a higher average than the hexaploids, but on account of the great variation among the hexaploids the difference was not significant. Also in the overwintering the hexaploids varied very much and their superiority over the tetraploids was consequently not substantiated.

7. DRY MATTER CONTENT, SUGAR CONTENT, AND OSMOTIC PRESSURE IN THE LEAVES.

During the winters 1937—38 and 1938—39 a series of investigations were carried out concerning dry matter content, sugar content, and osmotic pressure in a number of standard varieties and also in lines belonging to the different series of crosses. The previous experiments performed by ÅKERMAN (1927 a) and others in wheat postulate a general positive correlation between the dry matter content, sugar content, and osmotic pressure of different *vulgare* varieties on one side and their

TABLE 28. *Dry matter content, sugar content, and osmotic pressure in the standard varieties.*

Hardiness group	Variety	Resistance values in the freezing trials 37/38	38/39	Average [1]	Dry matter content, % of wet weight	Sugar content % of dry weight	Sugar content Sammet = 100	Osmotic pressure, mol cane sugar Dec. 37	Jan. 38	Febr. 39	Average
Vulgare:											
I	Sammet ...	3,6	4,1	3,9	21,3	21,7	100	0,64	0,59	0,60	0,61
II	Gluten	4,0	—	(3,8)	19,0	17,3	80	0,53	0,54	0,57	0,55
IV	0984 d	3,6	3,2	3,4	18,0	13,2	61	0,46	0,52	0,59	0,52
»	Ankar I ...	3,3	—	(3,1)	17,1	14,6	67	0,43	0,56	0,57	0,52
»	Sol III	3,2	—	(3,0)	17,0	14,1	65	0,51	0,54	0,58	0,54
V	0761	3,0	—	(2,8)	18,6	13,8	64	0,55	0,52	0,59	0,55
VIII	Wilhelmina	2,4	1,4	1,9	18,9	12,1	56	0,44	0,52	0,49	0,48
Turgidum:											
VI	Dinurum ...	3,2	2,7	3,0	17,4	11,4	53	0,42	0,46	0,49	0,46
VII	Red Rivet ..	2,6	1,6	2,1	19,6	12,1	56	0,45	0,44	0,51	0,47
IX	Compositum	2,2	1,3	1,8	17,6	12,3	57	0,43	0,45	0,53	0,47
Correlation with the average resistance in the freezing trials, r =					+ 0,444	+ 0,743	—	—	—	—	+ 0,766
t =					$1,40^{00}$	3,14*	—	—	—	—	3,37**

[1] See text.

TABLE 29. *Dry matter content, sugar content, and osmotic pressure in hexaploid and tetraploid lines of the 6000- and 8000-series.*

Line	Resistance values in the freezing trials			Dry matter content, % of wet weight	Sugar content, % of dry weight	Osmotic pressure, mol cane sugar			
	37/38	38/39	Average			Dec. 37	Jan. 38	Febr. 39	Average
Hexaploids:									
6000-series; 6064	3,3	2,8	3,1	19,2	13,0	0,49	0,48	0,54	0,50
6025	3,3	2,3	2,8	17,0	13,2	0,40	0,48	0,54	0,47
6098	3,2	2,2	2,7	19,7	12,6	0,44	0,50	0,54	0,49
6071	3,5	1,7	2,6	18,7	12,7	0,45	0,50	0,58	0,51
6177	2,7	2,5	2,6	17,6	10,9	0,43	0,49	0,55	0,49
6009	3,3	1,7	2,5	16,3	14,6	0,42	0,49	0,57	0,49
6017	2,7	2,3	2,5	19,4	12,6	0,45	0,44	0,53	0,47
6241	2,8	2,0	2,4	16,5	12,0	0,50	0,50	0,55	0,52
6163	2,8	—	—	17,7	12,3	0,46	0,48	—	—
6147	2,7	—	—	16,9	12,4	0,40	0,49	—	—
8000-series; 8077	3,7	2,5	3,1	18,7	15,6	0,61	0,50	0,60	0,57
8086	3,0	3,2	3,1	17,7	15,4	0,50	0,52	0,55	0,52
8094	2,7	3,0	2,9	18,2	14,1	0,48	0,50	0,58	0,52
8022	3,3	2,3	2,8	17,6	11,6	0,45	0,49	0,56	0,50
8071	3,2	2,0	2,6	17,9	19,1	0,51	0,46	0,55	0,51
8036	3,2	1,8	2,5	19,4	14,3	0,44	0,52	0,51	0,49
8046	2,7	2,3	2,5	19,4	12,8	0,49	0,52	0,53	0,51
8042	2,7	1,5	2,1	17,6	11,8	0,42	0,47	0,48	0,46
8125	3,3	—	—	19,2	11,6	0,53	0,54	—	—
Tetraploids:									
6000-series; 6194	1,8	3,3	2,6	20,1	11,5	0,43	0,50	0,55	0,49
6153	2,5	2,0	2,3	18,1	15,5	0,46	0,51	0,50	0,49

	6153	2,5	2,0	2,3	18,1	15,5	0,46	0,51	0,50	0,49
	6090	2,0	2,0	2,0	20,5	11,9	0,45	0,43	0,53	0,47
	6207	2,3	1,5	1,9	20,2	14,2	0,41	0,53	0,55	0,50
	6195	1,8	2,0	1,9	20,1	11,2	0,43	0,47	0,55	0,48
	6145	1,8	1,7	1,8	23,2	8,4	0,41	0,43	0,57	0,47
	6136	1,3	1,8	1,6	22,6	8,1	0,41	0,44	0,56	0,47
	6225	1,8	—	—	20,8	12,0	0,45	0,47	—	—
	6217	—	1,2	—	19,5	12,3	0,46	0,51	0,55	0,51
	6041	—	—	—	20,6	10,8	0,41	0,50	—	—
8000-series;	8074	3,7	2,5	3,1	20,1	15,1	0,48	0,53	0,55	0,52
	8069	3,3	2,7	3,0	25,3	15,0	0,55	0,51	0,59	0,55
	8083/84	2,7	2,7	2,7	20,9	14,8	0,46	0,52	0,54	0,51
	8047/48	2,7	2,4	2,6	21,5	15,4	0,51	0,48	0,57	0,52
	8038/39	2,7	2,3	2,5	19,7	16,5	0,54	0,52	0,58	0,55
	8056/57	2,8	2,0	2,4	22,2	12,2	0,49	0,52	0,57	0,53
	8028	2,3	2,2	2,3	19,9	13,7	0,48	0,48	0,54	0,50
	8099	1,7	2,5	2,1	20,8	12,4	0,46	0,47	0,53	0,49
	8035	2,2	1,7	2,0	20,7	13,3	0,48	0,49	0,53	0,50
	8118	1,7	1,8	1,8	20,1	12,5	0,47	0,47	0,55	0,50
Averages:										
6000-series;	6x	3,03	2,19	2,65	17,90	12,63	0,444	0,485	0,550	0,493
»	4x	1,91	1,94	2,01	20,57	11,59	0,432	0,479	0,545	0,485
8000-series;	6x	3,09	2,33	2,70	18,41	14,03	0,492	0,502	0,545	0,510
»	4x	2,58	2,28	2,45	21,12	14,09	0,492	0,499	0,555	0,517
All hexaploids		3,06	2,26	2,68	18,14	13,29	0,467	0,493	0,548	0,501
» tetraploids		2,28	2,13	2,27	20,85	12,84	0,462	0,489	0,551	0,503
Difference 6x—4x		+ 0,78	+ 0,13	+ 0,41	— 2,71	+ 0,45	+ 0,005	+ 0,004	— 0,003	— 0,002
Error ±		0,164	0,168	0,125	0,422	0,668	0,015	0,009	0,009	0,009
t = ...		4,76***	0,77^{000}	3,28**	6,42***	0,67^{000}	0,33^{000}	0,44^{000}	0,34^{000}	0,23^{000}
Correlation with the average resistance in the freezing trials, r = ...					— 0,278	+ 0,528	—	—	—	+ 0,554
t = ...					1,61^{00}	3,46**	—	—	—	3,71***

resistance to cold on the other side, if the investigations are carried out on plants of the winter-stage hardened at low temperature. During the hardening process the content of sugar in the leaves is increased to a different degree in the varieties. The object of my own investigations was to test the validity of these rules in the *vulgare* × *turgidum* progenies.

The determinations of dry matter and sugar content were performed in the winter 1937—38. The osmotic pressure was determined on three occasions, viz. December 1937, January 1938, and February 1939. In the trials seven *vulgare* and three *turgidum* standard-varieties occurred (Table 28). These varieties are ranked according to their resistance in the freezing trials. The third column of the resistance values, marked »Average», gives the real average values of the two years for the varieties Sammet, 0984 d, Wilhelmina, and the three *turgidum* varieties. For the other varieties, which were not included in the freezing trials of 1938—39, »average values» (given within brackets) are calculated from the differences in other years between the variety in question and 0984 d as standard (Table 24).

The dry matter content in per cent of the wet weight was highest in the hardiest variety Sammet, but the correlation with the average resistance-values was otherwise less good, and proved non-significant. The varieties Red Rivet and Wilhelmina gave surprisingly high values, while Ankar I and Sol III behaved in the reverse way.

The sugar content was determined by BOBECK's (1928) modification of the HAGEDORN-JENSEN micro-method. In the table the sugar content is given in per cent of the dry matter, and the relative value with Sammet as standard is also recorded. The agreement with ÅKERMAN's results (Table 22) is comparatively good, especially in the hardiness groups I—IV. The *turgidum* varieties seem to behave in a different manner. The correlation between sugar content and cold-resistance among the standard varieties as a whole is, however, significant.

At the determination of the osmotic pressure the plasmolysis with cane sugar was studied in the epidermal cells of winter leaves. The material was sown in plots close to the laboratory in order to avoid long transports and drying of the leaves. The observation of plasmolysis was facilitated by addition of neutral-red to the plasmolyte in a concentration of 0,05 ‰. The concentration intervals between the cane-sugar solutions were 0,05 mol, and averages were calculated from the determinations in at least five leaves from different plants. In the first

series of determinations, December 1937, the two *vulgare* varieties 0984 d and Ankar I showed astonishingly low values, but in the other two series this was not repeated. The average results of the table present a falling scale, and the correlation to the cold-resistance proved significant.

The same physiological conditions were also studied in 39 lines of the 6000- and 8000-series (Table 29), 19 of which were hexaploid and 20 tetraploid. The average results, given at the foot of the table, agree only partly with the conditions in the standard varieties. The same significant, positive correlation was established between sugar content and cold-resistance and also between osmotic pressure and cold-resistance. The correlation between dry matter content and cold-resistance was, however, negative but non-significant.

Although the hexaploid lines showed better average values of cold-resistance than the tetraploid lines, the tetraploids had significantly higher dry matter content, the difference being 2,71 %. With respect to the sugar content the hexaploids had a 0,45 % higher average, but no significance was obtained. The osmotic pressure, finally, demonstrated the curious situation that practically no difference existed between the average of hexaploid and that of tetraploid lines, in spite of the positive correlation between osmotic pressure and cold-resistance and the superiority of the hexaploids in cold-resistance.

When the leaves were taken to the laboratory for the determination of dry matter and sugar content in December, 1937, it was observed that some of the tetraploid lines were partly damaged by cold (viz. 6041, 6136, 6217, and 6225). For the investigations only quite undamaged leaves were used, and there is no reason to assume that the damages could influence the laboratory investigations, since these four lines do not give any deviating results.

8. VISCOSITY AND PERMEABILITY OF PLASM.

In order to attack the problem of the causes of different cold-resistance in my wheat material from another point of view some experiments on the determination of plasm viscosity and permeability were carried out during the winter 1938—39 in co-operation with Dr. GÖSTA ANDERSSON.

KESSLER (1935), studying leaves of *Sempervivum* and other plants after centrifuging at a certain velocity and for a certain length of time, found that leaves which had been hardened were relatively unaffected, while non-hardened leaves showed a characteristic clustering of the

chloroplasts. The viscosity of the plasm, which regulates the resistance to clustering, was consequently higher in the hardier plants.

The same method was applied by me to wheat leaves from seven standard varieties of *vulgare* and one of *turgidum*, representing widely

TABLE 30. *Determination of plasm viscosity by means of centrifuging.*

Hardiness group	Variety	Experiment A Revs. per minute		Experiment B Revs. per minute		
		1650	2000	1200	2000	2600
I	Sammet	— —	—	0	±	±
II	Minhardi	— —	— —	— —	— —	—
»	Svea II	— —	—	0	0	0
IV	Sol III	— —	±	— —	+	++
V	Pansar II	+	+	0	0	0
VII	Carsten V	+	++	++	++	++
VIII	Wilhelmina	++	++	+	++	++
IX	Compositum	0	0	++	++	++

Explanations:

— — = completely unaffected cell content.
— = slight traces of disturbance.
± = single cells with clustered chloroplasts.
\+ = more common clustering of the chloroplasts.
\+ + = chloroplasts in all cells tightly clustered.
0 = not investigated.

different hardiness groups. The results given in Table 30 show that also in wheat the viscosity of the plasm seems to be correlated to cold-resistance. Centrifuging was continued for 15 minutes in both experiments.

Repeated attempts to apply the same method to a number of 6000- and 8000-lines unfortunately failed on account of the insignificant differentiation between the lines.

The permeability of the plasm was studied by means of HÖFLER's (1918 a, b, 1936) »plasmometrische Methode». The technique was studied by me during a visit to Vienna in March, 1938, and in the spring of 1939 some experiments were performed at Svalöf, mainly with the two varieties 0984 d and Red Rivet, but also with two hexaploid and two tetraploid 6000-lines. Epidermal cells were plasmolysed in hypertonic solutions (1,0-normal) of glycerine and urea, and the velocity of permeation was observed by means of repeated measurings of the expansion of the plasmolysed protoplast. No clear differences were observed between the different varieties and lines, and the only result obtained was that in most experiments glycerine seemed to permeate

TABLE 31. *Time of heading, plant height, and resistance to diseases in the standard varieties.*

Variety	Time of heading				Plant height, cm				Black chaff		Yellow rust	Black stem rust
	1936	1937	1938	1939	1936	1937	1938	1939	1938	1939	1939	1938
I. *Hexaploids*:												
0984 d	$^{24}/_{6}$	$^{24}/_{6}$	$^{20}/_{6}$	$^{21}/_{6}$	96	116	137	109	8,6	8,2	5,2	7,0
Sol III	—	— 3	— 4	— 3	—	109	135	116	8,2	8,3	4,2	7,2
Ankar I	—	+ 1	+ 1	— 2	—	113	135	120	8,0	8,0	3,0	7,5
Pudel	—	± 0	± 0	— 2	—	115	143	108	7,7	7,3	4,0	8,0
Gluten	—	— 5	— 4	— 4	—	105	137	100	7,0	8,5	3,0	7,5
0761	—	+ 1	+ 2	+ 1	—	110	138	120	8,0	8,0	4,5	5,3
01201	—	—	— 4	— 4	—	—	125	110	7,0	8,0	4,5	7,0
Carsten V	—	—	— 5	— 3	—	—	130	110	—	8,0	5,0	—
Sammet	—	—	— 3	— 1	—	—	140	120	7,0	7,0	4,0	5,0
E. Squarehead II	—	—	— 3	— 1	—	—	130	110	8,0	9,0	1,0	7,0
Bearded Club	—	—	— 7	— 3	—	—	110	95	8,0	8,0	4,0	8,0
White Spelt	—	—	— 4	— 2	—	—	135	125	9,0	8,5	4,0	7,0
Blue Spelt	—	—	— 8	— 9	—	—	125	105	8,0	7,0	1,0	6,0
VVT 6009/11	—	—	— 1	— 1	—	—	135	120	9,0	8,7	6,0	7,5
VVT 6051/64	—	—	— 4	— 4	—	—	105	100	6,7	7,0	6,0	6,7
VVT 6103/104	—	—	— 7	—	—	—	—	—	6,0	—	—	6,0
VVT 6240/244	—	—	± 0	— 1	—	—	145	105	7,0	8,0	6,5	8,0
SVT 6163/167	—	—	— 3	— 3	—	—	140	130	8,0	8,0	5,5	8,0
SVT 6179/185	—	—	— 4	— 5	—	—	135	120	8,0	8,0	2,0	8,0
II. *Tetraploids*:												
Red Rivet	± 0	+ 4	— 1	— 2	122	129	146	135	6,9	8,1	7,9	8,1
Compositum	—	+ 2	— 5	— 4	—	120	139	130	7,0	6,7	7,7	7,4
TVT 6214/217	—	—	+ 12	+ 10	—	—	110	100	5,0	5,0	9,0	9,0
TcVT 6221	—	—	+ 11	+ 11	—	—	90	95	6,0	8,5	6,5	9,0

considerably swifter than urea. In this connection it is worth mentioning that GREIS (1940), who tried to investigate the permeability in autotetraploid and diploid barley with the same method, reports that he had to give up the experiments on account of too great variation in the results obtained. The method seems consequently less applicable to the Gramineae.

9. TIME OF HEADING AND PLANT HEIGHT IN THE FIELD TRIALS.

Time of heading. — In the discussion on the effect of artificially induced polyploidy the time of development plays an important rôle. In practically all cases the autotetraploids are later in developing and ripening than the diploid original material.

TABLE 32. *Time of heading in the 6000-series 1936—1939.*

0984 d = 0 Days earlier = — Days later = +	1936		1937		1938		1939	
	6x	4x	6x	4x	6x	4x	6x	4x
— 13 — — 8	9	—	5	—	5	—	1	—
— 7 — — 2	44	1	53	—	22	—	9	1
— 1 — + 4	36	24	36	6	7	10	3	6
+ 5 — + 10	2	10	12	11	4	6	—	2
+ 11 — + 16	—	5	1	12	—	5	—	5
+ 17 — + 22	—	4	—	3	—	—	—	—
+ 23 — + 28	—	1	—	7	—	—	—	—
Total:	91	45	107	39	38	21	13	14
Average:	—2,3	+5,8	— 1,6	+12,3	—2,6	+5,2	—3,7	+5,8
Differences:	—8,1	±1,03	—13,9	± 1,26	—7,8	±1,28	—9,5	±1,83
t =	7,86***		11,03***		6,09***		5,19***	

The progenies of *Triticum vulgare* × *turgidum* show quite different conditions since the lines of the higher chromosome stage, the hexaploids, are on an average distinctly later in their development rhythm than the lines of the lower stage, the tetraploids. As a standard of the development the time of heading has been used because it is easier to make observations of this stage, whereas the ripening time is considerably more difficult to observe, being more influenced by accidental outer conditions, for instance, rainfall or especially warm and forcing weather.

In Table 31 a number of standard varieties are collected. As seen there, 0984 d and Red Rivet, on an average, show their heads about the same day. In the 6000-series derived from the cross between these two varieties (Table 32) the distribution of the date of heading is, however,

very wide. In all the four years 1936—39 the tetraploid lines were significantly later than the hexaploids, if the averages are compared. (In 1940 the winter-damages made the observations in the field plots rather capricious, and therefore this year has been omitted from the tables.) The extremes are represented by some *vulgare*-like hexaploids, which are nearly two weeks earlier than 0984 d, and by some *turgidum*- or *contractum*-like tetraploids nearly four weeks later than 0984 d. A few late hexaploids are also found, shooting their heads about a week later than 0984 d, these lines usually being *speltoides*. Several *speltoides* lines are, however, earlier than 0984 (see Table 31). Also in the 8000- and 9000-series (Table 33) the tetraploid lines are significantly later than the hexaploid ones in spite of very small differences between the parents. The *turgidum* variety Compositum is usually a couple of days earlier than 0984 d, but in late years Compositum — and also Red Rivet — are often a little later than 0984 d, at least at the ripening. In the 9000 B-series the tetraploid parents are often considerably later

TABLE 33. *Time of heading in the 8000- and 9000-series 1937—1939.*

0984 d = 0 Days earlier = — Days later = +	8000-series						9000 A-series				9000 B-series			
	1937		1938		1939		1938		1939		1938		1939	
	6x	4x	6x	4x	6x	4x	6x	4x	6x	4x	6x	4x	6x	4x
— 13 — — 8	3	—	2	—	2	—	—	—	1	—	—	—	—	—
— 7 — — 2	6	1	31	13	24	6	14	1	20	8	23	—	26	—
— 1 — + 4	25	16	12	29	12	23	13	11	10	7	18	4	16	9
+ 5 — + 10	1	16	—	11	—	9	—	8	—	7	—	7	—	3
+ 11 — + 16	—	1	—	1	—	3	—	1	—	3	—	—	—	1
+ 17 — + 22	—	—	—	—	—	—	—	2	—	—	—	1	—	—
Total:	35	34	45	54	38	41	27	23	31	25	41	12	42	13
Average:	—0,7	+4,3	—3,7	+1,5	—2,6	+2,2	—1,9	+5,0	—2,4	+2,8	—2,9	+6,6	—2,3	+2,9
Differences:	—5,0	±0,85	—5,2	±0,70	—4,8	±0,76	—6,9	±1,36	—5,2	±1,20	—9,5	±1,58	—5,2	±0,94
t =	5,88***		7,43***		6,32***		5,06***		4,33***		6,01***		5,53***	

than the hexaploid parents, but the difference between 6x and 4x derivatives is not more marked here than in the 6000-series, where the parents are equally early. The hexaploids in all series are on an average 0,7—3,7 days earlier than the standard 0984 d, while the tetraploids are 1,5—12,3 days later than 0984 d.

Plant height. — This is no real physiological character, but since the observations on plant height were carried out on the same material as was used for physiological investigations of different kinds it seems appropriate to insert them here.

Red Rivet is usually about 20 cms higher than 0984 d (Table 31). In the 6000-series, however, the differences between the lines are much more pronounced, the extremes being represented by *speltoides* with about 160 cms and *contractum* with only about 40 cms. Although

TABLE 34. *Plant height in the 6000-series 1936—1939.*

Height, cm	1936		1937		1938		1939	
	6x	4x	6x	4x	6x	4x	6x	4x
180—155	—	—	2	—	3	—	—	—
150—125	2	—	18	1	18	2	3	1
120— 95	59	13	75	19	—	11	9	5
90— 65	29	20	12	11	—	1	1	7
60— 35	1	11	—	7	—	—	—	1
Total:	91	44	107	38	21	14	13	14
Average:	100,2	81,1	112,9	90,5	142,9	112,1	118,5	92,1
Differences:	+19,1	±3,32	+22,4	±4,05	+30,8	±4,87	+26,4	±6,53
$t =$	5,75***		5,53***		6,32***		4,04***	

the tetraploid parent is higher than the hexaploid, the 6x derivatives show a significantly higher average than the 4x lines in the four years (Table 34). The highest *speltoides* lines, 6174—6178, were all derived from the same F_3 plant. The F_4 plot from this plant was so high in 1935 that chromosomal aberrations of some kind or other were suspected. The chromosome counts gave, however, only $2n = 42$ (see Table 17).

In the 8000-series the tetraploid parents were also higher than the hexaploid ones, but the 6x lines from the crosses were significantly higher than the 4x lines (Table 35). In the 9000 A-series, derived from the back-crosses, the difference between 6x and 4x was also established. In the 9000 B-series, on the other hand, where the parents (6000-lines and varieties from different species) were highly variable in length (Table 31), no significant difference was obtained between 6x and 4x (Table 35).

TABLE 35. *Plant height in the 8000- and 9000-series 1938—1939.*

Height, cm.	8000-series 1938		8000-series 1939		9000 A 1939		9000 B 1939	
	6x	4x	6x	4x	6x	4x	6x	4x
180—155	3	1	—	—	—	—	—	—
150—125	35	12	17	5	8	3	16	3
120— 95	1	24	20	24	21	15	23	10
90— 65	—	5	1	12	2	7	2	—
Total:	39	42	38	41	31	25	41	13
Average:	139,7	116,4	124,2	104,4	116,8	104,4	119,8	118,5
Differences:	+23,3	±3,60	+19,8	±3,51	+12,4	±3,91	+1,3	±4,19
t =	6,47***		5,64***		3,17**		0,31 000	

10. RESISTANCE TO BACTERIUM TRANSLUCENS VAR. UNDULOSUM, PUCCINIA GLUMARUM, AND P. GRAMINIS.

Bacterial black chaff. — Attacks of this disease on wheat are reported especially from America (BAMBERG, 1936). Black, dark brown, or dark violet spots on the empty glumes and on the upper part of the stems are developed at the period just before the ripening. The disease is caused by *Bacterium translucens* var. *undulosum*, and attacks have also been observed at Svalöf on several occasions (FRÖIER, 1938). Differences in resistance have been observed in the Swedish *vulgare* varieties.

Especially evident symptoms of black chaff were often noted in my interspecific wheat hybrids, but usually the attacks of the disease were only sporadical and irregular in occurrence. In the years 1935, 1938, and 1939, however, the attacks were so general that the damages admitted of being graded. In 1935 an estimation of the attacks was carried out in the F_1 material and the corresponding parental varieties. The following results were obtained (10 = completely undamaged, 1 = extremely hard attack): Cross No. *5* — Sol III 8,6, Compositum 8,0, F_1 7,5; No. *6* — Sol III 8,6, Red Rivet 5,5, F_1 7,6; No. *7* — Ankar I 8,3, Red Rivet 5,0, F_1 7,4; No. *8* — Pudel 6,0, Red Rivet 4,0, F_1 7,0; No. *9* — Gluten 6,4, Red Rivet 4,6, F_1 5,0; No. *10* — 0761 10,0, Red Rivet 4,5, F_1 5,0; No. *11* — 0984 d 8,4, Red Rivet 4,0, F_1 4,7. The *vulgare* parents were consequently more resistant than the *turgidum* parents in the different crosses, and the F_1 plants usually proved intermediate.

Among the parental varieties in 1938 and 1939 (Table 31) all the *vulgare* varieties were relatively resistant. The *turgidum* varieties Red Rivet and Compositum were attacked slightly more, but were not severely damaged by the disease. The dark spots in these two varieties

were especially localized to the empty glumes and the awns, just as in the *vulgare* varieties. Some of the 6000-lines used as parents in the 9000 B-series, viz. VVT 6103/104, TVT 6214/217, and TcVT 6221, were transgressively susceptible and especially the line TVT 6214/217 had black spots not only in the head but also on the upper part of the stem.

In the 6000-, as well as in the 8000- and 9000 A-series the hexaploids were on an average a little better than the tetraploid lines, but only in the 8000-series in 1938 was the difference significant. In the 9000 B-series there is a reverse but non-significant difference. Altogether the resistance seems relatively independent of the chromosome number. The variation was very wide in both groups.

Yellow rust. — Studies on the inheritance of resistance and susceptibility to *Puccinia glumarum* have been made by a great number of investigators. BIFFEN (1905) assumed that in crosses within the *vulgare* wheats and also between *vulgare* and *turgidum* wheats the susceptibility was dominant in F_1 and that F_2 gave 3 susceptible: 1 resistant. NILSSON-EHLE (1911) found, however, that the segregation in *vulgare* was usually more complicated on account of the co-operation of several genes. In more recent times the discovery of different biological races of yellow rust has thrown new light upon the sometimes capricious attacks of this disease. The tetraploid species are usually comparatively resistant to most races of yellow rust, and pentaploid wheat crosses have therefore been performed in order to obtain hexaploid resistant varieties. Such types proved, however, to occur extremely rarely among the segregates. WATKINS (1930) has given a factorial explanation which is analogous to his *K*-formulae, presuming susceptible *vulgare* $= (pp)P'P'$ and resistant *turgidum* $= (PP)$. The exceptional hexaploid resistant types are explained by assuming occasional pairing between a *C*-chromosome with the *P*-factor and an emmer-chromosome.

In my own material the attacks of yellow rust in the field trials were only in one year, 1939, so generally distributed over the field that an estimation of the resistance could be performed. The *vulgare* parents were all attacked by the rust and the *compactum* and *Spelta* varieties also proved susceptible (Table 31). The two varieties Extra-Squarehead II and Blue Spelt were completely yellow in all leaves and also had yellow stripes on the empty glumes of the ears. Among the 6000-lines used as parents in the 9000 B-series the *vulgare* lines 6009/11, 6051/64, and 6240/244 were clearly better than their *vulgare* parent 0984 d. On the other hand, SVT 6167/185 was more susceptible than

TABLE 36. *Bacterial black chaff in the 6000-, 8000-, and 9000-series 1938—1939.*

Resistance value (10 = no attack)	6000-series 1938		6000-series 1939		8000-series 1938		8000-series 1939		9000 A 1939		9000 B 1939	
	6x	4x	6x	4x	6x	4x	6x	4x	6x	4x	6x	4x
10—9	7	3	—	—	2	—	3	6	9	6	2	1
8—7	17	6	13	13	39	31	35	30	21	14	38	12
6—5	11	9	—	—	4	17	—	1	1	4	2	—
4—3	2	2	—	1	—	6	—	4	—	1	—	—
2—1	1	1	—	—	—	—	—	—	—	—	—	—
Total:	38	21	13	14	45	54	38	41	31	25	42	13
Average:	6,95	6,10	7,85	7,36	7,51	6,41	8,00	7,54	8,00	7,60	7,74	7,92
Differences:	+0,85	±0,502	+0,49	±0,373	+1,10	±0,220	+0,46	±0,246	+0,40	±0,294	—0,18	±0,176
t =	$1{,}69^{0}$		$1{,}31^{00}$		$5{,}00^{***}$		$1{,}87^{0}$		$1{,}36^{00}$		$1{,}02^{000}$	

TABLE 37. *Yellow rust in the 6000-, 8000-, and 9000-series 1939. Black stem rust in the 6000- and 8000-series 1938.*

Resistance value (10 = no attack)	Yellow rust 6000-ser.		Yellow rust 8000-ser.		Yellow rust 9000 A-ser.		Yellow rust 9000 B-ser.		Black stem rust 6000-ser.		Black stem rust 8000-ser.	
	6x	4x	6x	4x	6x	4x	6x	4x	6x	4x	6x	4x
10—9	3	5	—	6	—	—	—	—	4	17	—	5
8—7	8	9	12	28	—	10	1	8	32	4	20	42
6—5	2	—	16	6	9	11	10	5	2	—	21	5
4—3	—	—	6	—	18	4	21	—	—	—	3	2
2—1	—	—	4	1	4	—	10	—	—	—	1	—
Total:	13	14	38	41	31	25	42	13	38	21	45	54
Average:	7,62	8,36	5,42	7,34	3,87	6,00	3,55	6,77	7,45	8,81	6,27	7,52
Differences:	—0,74	±0,316	—1,92	±0,364	—2,13	±0,348	—3,22	±0,344	—1,36	±0,210	—1,25	±0,284
t =	$2{,}34^{*}$		$5{,}27^{***}$		$6{,}12^{***}$		$9{,}36^{***}$		$6{,}48^{***}$		$4{,}40^{***}$	

0984 d. The *turgidum* varieties Red Rivet and Compositum were both relatively resistant. Of the two tetraploid 6000-lines in Table 31, one, TVT 6214/217, was practically free from traces of yellow rust, while the other one, TcVT 6221, was intermediate between 0984 d and Red Rivet.

In all the different series of derivatives (Table 37) the tetraploid lines were significantly more resistant than the hexaploids. The variation was, however, great in both groups. In the 6x group three lines of the 6000-series, viz. SVT 6017, VVT 6147, and SVT 6177, seemed very resistant. On the other hand, several 4x lines of the 8000- and 9000-series proved more susceptible than their most susceptible parent.

Black stem rust. — The inheritance of resistance to *Puccinia graminis tritici* seems to follow about the same lines as already described for *P. glumarum*. A great number of biological forms of the black stem rust are distinguished, and different genes seem to be responsible for the resistance to these forms (HARRINGTON and SMITH, 1929). In pentaploid crosses between resistant tetraploids and susceptible hexaploids a great number of plant breeders have tried to find resistant hexaploid derivatives. Their occurrence is extremely rare, but the varieties Marquillo and Hope, already mentioned in Chapter II: 3 of the present paper, furnish good examples.

In the harvested plants of my trials of 1938 the attacks of black rust were estimated. Of the *vulgare* standards only 0761 and Sammet, both belonging to the Swedish land-wheat type, were severely attacked (Table 31). Some of the hexaploid 6000-lines used as standards were quite resistant. All the tetraploid parents were very resistant. In the 6000- and also in the 8000-series the susceptibility seemed to vary considerably. On an average the tetraploids were significantly better. Among the hexaploid 6000-lines, four, viz. VVT 6104, VVT 6149, SVT 6177, and SVT 6178, seemed very resistant. Several of the tetraploid lines in the 8000-series were attacked to an astonishingly severe degree, especially TVT 8116 and TVT 8118.

IV. DISCUSSION.

At the studies in progeny generations of the species cross *vulgare* (6x) × *turgidum* (4x) and a number of other cross combinations within the genus *Triticum*, described in the foregoing chapters, several different problems have been involved. Most of them have already been discussed

in detail under the respective chapter headings. As to some other problems, however, and especially the inheritance of the species-distinguishing morphological characters and the physiological qualities of cold-resistance, vegetation period, and resistance to diseases, some further remarks and explanations will be added.

The species characters in Triticum. — From the morphological point of view the current *Triticum* species are best distinguished by the appearance of their empty glumes and rachis. The genetical background of these characters is formed by four different circumstances.

(1) The existence of polyploidy, splitting the genus into three species groups — diploids (2n = 14), tetraploids (2n = 28), and hexaploids (2n = 42).

(2) The differences between the three genomes are so great that allopolyploidy is postulated. On account of the affinities found the following genome formulae are established: diploids *AA*, tetraploids *AA . BB* (*Timopheevi AA . GG*), hexaploids *AA . BB . CC*.

(3) Each one of the genomes (*A*, *B*, and *C*) contains a special gene complex, which can appear in different multiple allelomorphs (named *K*, K^s, K^d, *k*) and is constituted by a number of completely linked genes. This *K*-complex regulates to an essential degree the form, keel, thickness and attachment of the empty glumes, the toughness or brittleness of the rachis, etc., and partly also the density and length of the spike, etc. The following *K*-formulae are established by WATKINS (1928, 1940): *dicoccum KK . K^dK^d*, *turgidum* and *durum KK . KK*, *vulgare KK . kk . K^dK^d*, *Spelta KK . K^sK^s . K^dK^d* (see also p. 310).

(4) Species with the same chromosome number and the same *K*-formula, e. g. *turgidum* and *durum*, are separated by other genes or gene complexes. It seems probable that some other pairs of species, e. g. *durum* and *polonicum* (BIFFEN, 1905; ENGLEDOW, 1920), *vulgare* and *compactum* (NILSSON-EHLE, 1911; KAJANUS, 1923; and others), where the difference mainly seems to be monofactorial, also belong to this category.

The formulae given by WATKINS have given a satisfactory explanation of the occurrence of 42-chromosomal *Spelta*-like forms, *KK . KK . K^dK^d* (in the present paper named *speltoides*, SVT), and 28-chromosomal *compactum*-like forms *KK . kk* (here named *contractum*, TcVT) in the progenies of *vulgare* × *turgidum* (V × T). In the cross experiments carried out by me the *K*-formulae have been tested in several different combinations. Special attention has been devoted to the three morphologically proximal 42-chromosomal types *Spelta* (S), *speltoides* out of

vulgare × *turgidum* (SVT), and homozygous speltoid mutant of A-type out of *vulgare* (SV), as well as to the two morphologically similar but in chromosome number different forms 42-chromosomal *compactum* (C) and 28-chromosomal *contractum* out of *vulgare* × *turgidum* (TcVT).

Of the three first-mentioned forms *Spelta* represents the most extreme stage. The glumes are especially hard and firm, and the rachis is brittle. *Speltoides* is markedly lax-headed, and furnished with thick tufts of hair on the rachis. In other respects it is very similar to the speltoid mutant, and KAJANUS (1923) and WATKINS apparently regard the two forms as identical though without any argumentation. The cross combinations S × SVT, S × SV, and SVT × SV have been carried out and studied by me. The continuous intermediate variation in the F_2 material of all the three crosses and the complete absence of segregates with loose, keelless glumes or dense ears are in accordance with the formulae S = *KK* . K^sK^s . K^dK^d, SVT = *KK* . *KK* . K^dK^d, and also seem to confirm that SV has the same formula as SVT. The appearance of A-speltoids in *vulgare* can easily be explained by mutation from *k* to *K* in the *B*-genome. Why these mutations arise so frequently remains, however, to be explained, and also the occurrence of speltoid mutants of B- and C-type and of compactoid forms in the speltoid series is left outside the present discussion.

The *K*-formula of *compactum* is not given by WATKINS. The cross *vulgare* × *compactum* gives a clear monofactorial segregation, and NILSSON-EHLE (1911) has explained this by assuming a dominant gene *C*, which is epistatic to the ear-lengthening genes. In the tetraploids, however, the *K*-complex is decisive for the appearance of dense and very short, completely *compactum*-like ears (*contractum KK*.*kk* as compared with common *turgidum KK* . *KK*), and consequently it might be expected that *compactum* differed from *vulgare* in the same complex in one of the genomes.

The results obtained from the crosses (*vulgare* × *turgidum*) F_1 × *compactum* and *compactum* × *contractum* together with the results of previous authors from *compactum* × *Spelta* are all satisfactorily explained by means of the assumption that *compactum* has the same *K*-formula as *vulgare* but differs from *vulgare* in the strongly epistatic *C*-factor of the *C*-genome.

The results of the cross (V × T) × C should be compared with the results of (V × T) × V. In the last-mentioned combination half of the F_1 plants give an F_2 with 3 *vulgare* and intermediate: 1 homozygous *speltoides*, while in the first-mentioned cross only $^1/_4 \times {}^1/_4 = {}^1/_{16}$ real

speltoides are obtained on account of the epistatic effect of the *C*-factor. At the assorting of the cross (V × T) × C it proved, however, very difficult to separate the *compactum—speltoides* plants from *vulgare*, since the *compactum* factor seems to efface all the *speltoides* characters nearly completely with the exception of the ear-lengthening. The density of the ears consequently varies from dense *compactum* to square-head and medium dense *vulgare*.

The cross C × TcVT gave only very dense forms in F_2, quite similar to the parental types. The appearance of *vulgare*-like plants in the subsequent generations deserves, however, special attention. The lack of the *C*-factor in the *C*-genome in these plants may be explained by natural crossing with *vulgare*, by mutation from *C* to *c*, or by exchange between the *C*-genome and one of the other two genomes. Natural crossing seems unlikely on account of the segregation observed in other characters. Since the *vulgare* plants appeared in five F_2 families out of 21, mutation also seems less probable. An exchange by means of autosyndesis (in the sense of pairing between chromosomes from the same gamete; LJUNGDAHL, 1924) has been supposed to happen casually for the *W*-factors that regulate the waxiness of the leaves in the cross *vulgare* × *turgidum* (WATKINS and CORY, 1931), rendering the formation of waxless *vulgare* possible. In the crosses *Spelta* × *aegilopoides* (6x × 2x) and *vulgare* × *durum* (6x × 4x) KIHARA and NISHIYAMA (1930) cytologically proved the affinity between the *A*- and *B*- and between the *B*- and *C*-genomes. This affinity enables autosyndesis in the crosses in question, and — as will be described later on — autosyndesis in crosses within the tetraploid wheat group is also known. In the present cross C × TcVT casual autosyndesis between homologous chromosomes of the *AB*- and *C*-genomes in F_1 would perhaps give an explanation of *vulgare*-like plants (>35 chromosomes) without the *C*-factor in F_3, but since no cytological investigations have been carried out on this point, and the segregation was not controlled in F_4, the question must be left open.

Irregularities in the chromosomal pairing also seem to occur in the cross *turgidum* × *contractum*. On account of the *K*-formulae and the ease with which *turgidum* (TVT) and *contractum* (TcVT) can be separated in F_2 of *vulgare* × *turgidum*, segregation in 3 T : 1 Tc was expected in F_2 of T × TcVT. Instead of this the variation proved continuous but mainly concentrated to the lax-eared side, so that only a small number of comparatively but not completely *contractum*-like plants were achieved. Similar phenomena are, however, known from several

other tetraploid wheat crosses. The first case was described by BIFFEN (1916) and dealt with the ear colour in *polonicum* × *turgidum*. The white colour of *polonicum* seemed to completely cover the gray of *turgidum* in F_1 and subsequent generations (»suppression»). In *polonicum* × *durum* ENGLEDOW (1920) showed that, in spite of the principally monofactorial segregation in length and type of empty glume, the pure *polonicum* type was not completely regained in the progeny of the cross (»shift»). In the cross *persicum* × *durum* VAVILOV and JAKUSHKINA (1925) found »shift», the *durum*-like apex of the keel being absent in the progeny. In the cross *turgidum* × *dicoccum* WATKINS (1940) reports that no segregation occurred in F_2, which, just as F_1, was most reminiscent of *dicoccum* as to glume type and also had the brittle rachis of *dicoccum* (»suppression»). On the basis of cytologically proved tri- and quadrivalent formations DARLINGTON (1927, 1928, 1931, 1937) has explained the cases mentioned above by means of autosyndesis and exchange between homologous *A*- and *B*-chromosomes. At »suppression» segregation in a »principal factor» fails to appear, and the whole progeny is constant and like F_1. At »shift», on the other hand, (e. g. in *durum* × *polonicum*) the principal factor segregates without disturbances in 3 : 1, but a »modifying factor» (*D* in *durum*), which is partially or completely dominant, does not segregate on account of autosyndesis. One of the parental types is therefore not reconstructed in the progeny of the cross (the *polonicum*-like plants will be *DD dd* just as F_1, and not *dd dd* as true *polonicum*).

The cross *turgidum KK . KK* × *contractum KK . kk* seems to offer a new example of »shift». The segregation of the *K*-factor is presumed to be smoothed out by autosyndesis in chromosomes carrying modifying factors, probably ear lengthening factors of some kind, which prevent the restitution of quite *contractum*-like types in F_2 and F_3. In this connection it will be mentioned that at the study of the cross combination *polonicum* × *turgidum* (cross No. *84, pol.* Martinari × *turg.* Dinurum, not included in Table 2) I found an F_2 segregation of 45 *turgidum*-like and intermediate : 18 *polonicum*. In this combination the characteristic glume form of *polonicum* was regained without disturbing »shift», which means that autosyndesis did not occur, at least not in the chromosomes where the factor for *polonicum* glumes or modifiers of this character were located.

It seems quite evident that the different »species» of the genus *Triticum* are not real species in a genetical sense, since many of them are only separated by a few characters, which segregate in a Mendelian

way. The phylogeny and the present relationships are, however, not yet so completely investigated that a definite revision of the species concept of the genus can be performed. On the whole, KAJANUS's (1927) suggestion that only three species should be maintained — corresponding to the three main groups and named *T. monococcum* L. *s. ampl.* (2n = 14), *T. acuminatum* KAJ. (2n = 28), and *T. obtusatum* KAJ. (2n = 42) — which are divided into subspecies, seems quite rational from the genetical point of view.

The inheritance of cold-resistance. — In the allopolyploids found in nature the hardiness is most commonly better than that of the related diploids. On the other hand, the majority of the experimentally produced auto- and allo-polyploids show lower cold-resistance or lower values in the qualities dry matter content and osmotic pressure, which are ususally correlated to the cold-resistance, than the diploid original forms. The discrepancies are to a certain extent elucidated, since it has been shown that artificial polyploids produced from different starting material can show very differing reactions (PIRSCHLE), so that sometimes a decrease, sometimes an increase, is obtained in the above-mentioned qualities. Furthermore, it has been shown in certain cases that the polyploids have a greater range of variation (KOSTOFF) and a greater physiological adaptability (GYÖRFFY). The discussion on the connection between polyploidy and physiology can consequently be divided into two main lines — a qualitative, comprising the gene succession from the original constituents, and a quantitative in which the reaction properties of all the accumulated gene quantity are considered (the polyploidy effect *per se*).

In the genus *Triticum* the hexaploids have on an average the best hardiness. *Vulgare, Spelta,* and *compactum* all contain winter forms with very good hardiness. Among the tetraploid species only *turgidum* can be considered as a true winter form. The hardiness of this species is in general only mediocre, and the northern limit of its cultivation area is in England, where the winter climate must be considered as very mild. It is, however, remarkable that the two diploid species, *monococcum* and *aegilopoides,* both of which are found in spring as well as winter form, in the latter case often show a very good hardiness. In Table 24 of the present paper *turgidum* is also represented by a couple of comparatively hardy varieties — of which especially Dinurum, which is clearly hardier than the most sensitive *vulgare* variety of the collection, Wilhelmina, can be mentioned. The diploids and especially *aegilopoides* are represented by very hardy forms. In the standard material used by

me no real correlation between chromosome number and cold-resistance consequently exists, and probably a more representative collection of wheat varieties of all the different species would give the same negative decision. It must be remembered, however, that wheat is a cultivated plant that was early surrendered to the protection, selection, and distribution of man. The genus thus has quite another appearance nowadays than in the earliest stages of cultivation and in the wild conditions of life. In the very last century plant breeding has been an especially effective agent in the metamorphosis of the different qualities of the wheat species, particularly within *vulgare*.

Genetic investigations on the winter-hardiness of the *vulgare* wheats carried out by NILSSON-EHLE (1912), ÅKERMAN (1923 a, 1924, 1927 a) and others have shown that the inheritance of this quality is multifactorial. This is also quite natural if we consider that winter-hardiness is a very complicated quality.

At the comparison of the cold-resistance of hexaploid and tetraploid derivatives of different cross series the following main results were obtained in the artificial freezing trials (Fig. 16). The 6000-series (V × T) — *vulgare* parent > 6x derivatives > *turgidum* parent > 4x derivatives. The 8000-series (V × T) — *vulgare* P > 6x > 4x > *turgidum* P on an average; in certain crosses the last two links were reversed. The 9000 A-series (F_1 × V, F_1 × T) — 6x > 4x. The 9000 B-series (V × VVT, T × TVT, S × SVT) — 6x > 4x. The 0000 A-series (V × T) — *turgidum* P > *vulgare* P > 6x > 4x; differences not significant. The 0000 B-series (V × T) — *vulgare* P > 6x > 4x > *turgidum* P. The observations of overwintering in the field plots, which were especially decisive in the hard winter of 1939—40, gave excellent confirmation of these results. In the series (6000, 8000, 0000 B) where the parents were hardy *vulgare* and sensitive *turgidum* varieties, the hexaploid lines have thus been more damaged than the *vulgare* parent but more resistant than the *turgidum* parent and the tetraploid lines. The relative resistance of the *turgidum* parent and the tetraploids has varied in the different varietal combinations. The tetraploids of 0984 d × Red Rivet (6000; 8000, cross No. *11*) have thus been distinctly more sensitive than Red Rivet; but in the other crosses, where the tetraploids were usually better than the *turgidum* P in the freezing trials, the differences were insignificant and sometimes contradicted by the field trials. In the 0000 A-series, where a comparatively hardy *turgidum* (Dinurum) was crossed with a less hardy *vulgare* (Wilhelmina), the differences between the parents and the hexaploids were very small. The tetraploids of this cross were inferior in

the freezing trial as well as in the field plots of 1939—40, though statistical evidence was not obtained.

In the following discussion of the probable reasons for the results obtained, the fact that the hexaploids from hardy *vulgare* × less hardy *turgidum* are on an average more hardy than the tetraploids will be considered first. The simplest explanation is that the *C*-genome in this case contains an especially rich stock of hardiness genes, which will thus be distributed to all the hexaploids. It seems, however, that a certain rôle must also be assigned to the polyploidy effect *per se* — the greater accumulation of hardiness genes possible in a higher ploid — since in the cross series 0000 A, where *turgidum* is the hardiest parent, the hexaploids also tend to show a higher average value than the tetraploids.

In order to explain the fact that the hexaploids have not on an average — and hardly in any single case either — achieved the hardiness of the *vulgare* parent, defective gene balance is assumed. Here I am not thinking of the circumstance that several of the hexaploid lines had not reached the full euploid stage on the occasion of the freezing, since it is quite evident from a comparison of the different resistance values that lines with a variation of 39—42 in the chromosome number are not more sensitive to cold than lines with constant $2n = 42$. As it is characteristic of the »increasing group» with $2n = 35$—42 that all the *C*-chromosomes must be represented — either as bivalents or as univalents — the lack of a few *C*-chromosomes including genes of hardiness will not influence the hardiness very much. In the pentaploid lines the conditions are quite different, and the hardiness is also very bad throughout.

The defective gene balance in the newly formed derivatives of the pentaploid cross is also visualized in the tetraploids, which are usually less hardy than the *turgidum* parent. Another phenomenon that is to be ascribed to the unbalance is the poorer germination in the hexaploid lines and especially in the tetraploid lines as compared with the germination of the parents.

The explanations of the hardiness conditions in the 6000-, 8000-, and 0000-series are corroborated by the behaviour of the 9000-series. In 9000 A, derived from back-crosses, as well as 9000 B, derived from crosses between 6000-lines and varieties of different species, the gene balance seems to be improved in so far that the lines approximate the parents with equal chromosome number in hardiness and also in germination.

Experiences from practical plant breeding point in the same direction, viz. that the genes must be balanced to produce a valuable in-

dividual. The balance can be described as a »total gene-interaction» in the fulfilment of the different physiological reactions — cf. RASMUSSON's (1933) interaction hypothesis for single quantitative characters. Every breeder knows that a certain variety with excellent practical properties will often prove a »bad parent», giving rise to nothing but disappointments when used in different cross combinations. It seems that the interaction of the genes is so delicately built up in such cases that all exchanges are deteriorating. In such complicated physiological qualities as the resistance to cold it is not remarkable that exchanges of genes between the *AB*-genomes of *vulgare* and those of *turgidum* lead to disturbances of the gene interaction. The genomes in question are probably homologous in a phylogenetic sense, but have been selected and changed in different climate and other outer conditions. The parental varieties in the crosses are especially selected plus-variants with good gene balance, but are very distant in their composition. Crosses between such parents seldom lead to direct practical breeding results, since the valuable types will be very few in the abundance of unbalanced combinations. Similar cases of unbalance are found in most gene mutations and certainly also in the artificially induced auto- and allo-polyploids. Selection and systematic crossing can, however, restore the balance and finally bring practical results.

The vegetation period. — In the cross *vulgare* × *turgidum* the two parents usually headed about simultaneously. The tetraploid derivatives were, however, on an average considerably later than the hexaploid derivatives. In the different series this was especially pronounced in 6000, where the tetraploids were 8—14 days later than the hexaploids.

In most polyploids the higher stages are later than the lower ones, but here the conditions are the reverse. The explanation seems to be multifactorial gene actions. If *vulgare* has genes for early development in the *C*-genome but genes for late development in the *AB*-genomes, while *turgidum* has an intermediate *AB*-constitution as to earliness, the hexaploid derivatives will show precocity and the tetraploids delay.

The resistance to diseases. — The relations between the parental species were different for black chaff and for the rust diseases. As regards black chaff *vulgare* was less attacked and the hexaploid derivatives also showed a clear tendency towards better resistance than the tetraploids. The *C*-genome seems to contain some extra genes for resistance. The existence of single extremely susceptible hexaploids indicates, however, that genes for this character are also located in the other genomes, and that the number of genes is probably great.

As to the yellow rust and black stem rust, the general experience is that the hexaploid species are more susceptible than the tetraploid ones, and this was also the case here. WATKINS has explained the inheritance of resistance in pentaploid crosses by assuming resistance and susceptibility genes in all the three genomes. The *C*-genome of *vulgare* seems to contain dominant genes for susceptibility, and the resistant hexaploid derivatives that sometimes arise are explained as the result of exchange between *C* and *AB*. In my own material this effect of the susceptibility genes of the *C*-genome was very evident as regards both rust species. Single positive transgressions were observed among the hexaploids but also negative ones among the tetraploids.

Neither in the resistance to diseases nor in the time of heading were any strong effects observed of the defective gene interactions in the descendants, just the opposite of the situation in cold-resistance. This indicates that cold-resistance is the most complicated quality, being the result of several co-operative genes and long physiological processes. Weak plants with disturbed balance have less possibility of hardening themselves and overcoming the damages of cold, but on the other hand such plants are not especially sensitive to, for instance, rust attacks, which are more likely to afflict the most vigorous plants. The selective importance of cold-resistance is also much more pronounced in northern latitudes than the resistance to rust. The old Swedish land wheats with their pronounced cold-resistance but susceptibility to rust attacks are good examples of this importance.

V. SUMMARY.

(1) The present investigations comprise a great number of crosses between different species of the genus *Triticum* and derivatives of such combinations.

(2) The seed setting of the reciprocals of artificial crosses between *vulgare* ($2n = 42$) and *turgidum* ($2n = 28$) was best when the high-chromosomal parent was used as female, and the same result was also obtained in other pentaploid crosses.

(3) The germination and viability of the F_1 plants in *vulgare* $\times$ *turgidum* and other pentaploid crosses was also best when the hexaploid parent was female.

(4) Hybrid vigour was observed in the F_1 plants of *vulgare* $\times$ *turgidum*.

(5) The chromosome numbers of F_2 in *vulgare* $\times$ *turgidum* showed

a distribution similar to that observed in other pentaploid wheat crosses by previous authors. On account of the peculiarities of the frequency curve intense elimination of univalents and sterile combinations seem to occur, as in *vulgare* × *durum*.

(6) After a number of generations the euploid chromosome numbers of the parents are restored in the progenies of *vulgare* × *turgidum*, but the constant tetraploid stage is obtained considerably quicker than the constant hexaploid stage.

(7) A constant 44-chromosomal plant was found in F_3, and some other aberrant numbers were also observed in different crosses.

(8) The segregation of beardness and ear pubescence in F_2 proved monofactorial.

(9) The occurrence of *speltoides* and *contractum* forms in the progeny of *vulgare* × *turgidum* is discussed.

Crosses between *Spelta*, *speltoides* and homozygous speltoid mutants (A-type) were carried out, and showed the close relationships of the three forms.

Contractum forms were crossed with the morphologically similar species *compactum*. In spite of the homogeneity of the F_2 type different factors are presumed to be responsible on account of the results obtained in other crosses.

(10) A new case of »shift» was observed in the tetraploid cross *turgidum* × *contractum*.

(11) Physiological investigations were performed in a great number of tetraploid and hexaploid lines of *vulgare* × *turgidum* and related crosses.

(12) Cold-resistance was tested in artificial freezing trials during several years.

When a hardy *vulgare* variety was crossed with a less hardy *turgidum*, the hexaploid derivatives were less hardy than *vulgare* but considerably hardier than the tetraploid derivatives. The last-mentioned lines were usually more sensitive than the *turgidum* parent.

When a comparatively hardy *turgidum* was crossed with a less hardy *vulgare*, the hexaploid lines also showed a tendency to better hardiness than the tetraploids, though not so good hardiness as the parents.

(13) The superiority of the hexaploids over the tetraploids is explained by the qualitative actions of a great number of hardiness genes distributed over all the three wheat genomes, and by a quantitative accumulation of such gene actions in the higher polyploid forms.

(14) The inferiority of the hexaploid lines to the *vulgare* parents, and usually also of the tetraploid lines to the *turgidum* parents, is assumed to be caused by disturbances in the »total gene-interaction», involving the whole gene mass and its regulation of the more complicated physiological processes.

(15) The results of the freezing trials and the assumption of disturbed balance in the new lines are confirmed by field observations on germination and overwintering. The balance seems to be improved in derivatives of back-crosses and similar combinations. In pentaploid lines the germination and winter-hardiness are especially defective, which is ascribed to cytological irregularities.

(16) Positive correlation was established between cold-resistance on one hand and sugar content and osmotic pressure on the other hand in the derivatives of *vulgare* × *turgidum*.

(17) In pure *vulgare* and *turgidum* varieties positive correlation was established between cold-resistance and viscosity of plasm. In *vulgare* × *turgidum* derivatives the centrifuging method gave no differentiation.

(18) The tetraploid derivatives are considerably later in development (time of heading) than the hexaploids on account of multifactorial segregation.

(19) The tetraploid lines are more resistant to yellow rust and black stem rust but more susceptible to bacterial black chaff than the hexaploid lines. The segregation seems to be multifactorial and especially connected with the *C*-genome.

(20) Unbalance in the »total gene-interactions» does not find such an evident expression in the developmental rhythm or in the resistance to diseases as it does in cold-resistance and germination ability. The first-mentioned characters are less complicated in a genetical and physiological sense and consequently less influenced by the total physiology of the plant.

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THE MECHANISM OF C-MITOTIC ACTION
OBSERVATIONS ON THE NAPHTHALENE SERIES

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INTRODUCTION.

THE discovery that colchicine could be used very successfully for the production of polyploids (BLAKESLEE, 1937) brought about a strongly increased interest in the cytological action of various chemicals. It had already been known for a long time that mitotic disturbances could be induced by chloral hydrate. Although these disturbances seem to be of the same nature as those caused by the colchicine, chloral hydrate is not suitable for the induction of polyploidy, because of its considerable poison effect. After the significance of the cytological effect of colchicine had been realized in 1937, very soon communications about a whole series of various substances with a similar action to that of colchicine began to appear. Among these substances, acenaphthene was the first to be discovered, and it must still be regarded as the most important one. Its c-mitotic effect was discovered by SHMUCK (1938), KOSTOFF (1938 a), and NAVASHIN (1938).

Acenaphthene has been used successfully by several workers for the production of polyploids. SHMUCK (1938) and KOSTOFF (1938 a) made the optimistic statement that acenaphthene might even be superior to colchicine, especially as its poisonous effect was very slight. Other authors, on the other hand, claimed that acenaphthene was relatively ineffective, as far as the production of polyploidy is concerned. The various opinions on this subject are reviewed by KRYTHE and WELLENSIEK (1942, pp. 40—41). The contradictory results obtained with acenaphthene may partly be due to different modes of treatment being employed by different workers. Acenaphthene is a hydrocarbon and it is therefore only very slightly soluble in water. KOSTOFF (1938 a, b) used a saturated water solution with an excess of undissolved crystals present. The necessity of an undissolved excess of solid substance is particularly stressed: ». . . saturated solution alone was not sufficient to induce chromosome doubling» (KOSTOFF, 1938 c, p. 753). JARETZKY and SCHENK (1940) comparing the effect of solutions with and without excess of crystals, however, found no appreciable difference, both

solutions being effective in Gramineae and ineffective in Leguminosae. The substance may also be applied in the form of solid crystals covering the inside of a test-tube, into which that part of the plant is introduced which is to be treated (KOSTOFF, 1938 c, d, e). The active principle in this kind of treatment is according to KOSTOFF the substance sublimating from the solid crystals. Vapour from the solid acenaphthene is recommended also by STRAUB (1941) in his practical hints for the production of polyploids.

A great many other substances with c-mitotic action, mainly benzene and naphthalene derivatives, had been discovered by SIMONET, GAVAUDAN, and SHMUCK and their collaborators. All these are applied in the same manner as acenaphthene. SHMUCK and GUSSEVA (1938, 1939, 1940 a, b) and SHMUCK and KOSTOFF (1939) dissolved the substances to be tested in ether, and prepared filter papers with the ether solution. After the evaporation of the ether, seeds were germinated in the filter papers.

The most detailed and systematic account on the different activities of naphthalene derivatives was made by SHMUCK and GUSSEVA (1939). They found that the introduction of certain radicals in the molecule of an active substance suppressed the c-mitotic effect.

The present investigation was primarily undertaken in order to test why acenaphthene and the other naphthalene derivatives are found to be less active than colchicine. Two possibilities seemed to be at hand: the substances *per se* may be less active than colchicine, or their lacking activity may be caused by practical difficulties at the treatments, due to their slight solubility in water.

Another problem taken up in the present investigation was suggested to us by the paper of SHMUCK and GUSSEVA (1939) just cited. When studying this paper we got the impression that a regularity was present which the writers themselves had perhaps not observed. The radicals which extinguished the c-mitotic activity were —OH, —NH_2, —CO_2H, —SO_3H, and sometimes also —NO_2 and haloid. Most of these are typical hydrophilic groups, about which it is known that as a rule they increase the solubility in water of a substance and decrease its solubility in lipoids. We therefore put the question: Is there a negative correlation present between the solubility in water of a substance and its c-mitotic activity? Such a correlation might be taken as evidence that the *partition of the substances between water and lipoids* played an important rôle in their mechanism of action. We decided to test this condition within different groups of related substances. In each substance the water

solubility and the threshold concentration of c-mitosis had to be determined. In the present paper we give the results of this work for a series of naphthalene derivatives. The results we obtained from some similar series among monocyclic substances are presented in another contribution (ÖSTERGREN and LEVAN, 1943).

I. EXPERIMENTAL PART.

1. METHOD.

In order to study the problems stated above it was first and foremost necessary for us to be able to carry out treatments with *exact dosages* of the substances. Throughout the investigation we had to work on a quantitative basis. Attempts to work quantitatively are so far very rare in the literature on c-mitotic substances.

In colchicine the lower threshold value for c-mitotic action was determined by NEBEL and RUTTLE (1938 a) for *Tradescantia* and by LEVAN (1938) for *Allium*. SHMUCK and GUSSEVA (1938) determined the minimum dosage of acenaphthene necessary for induction of c-mitoses in *Triticum* in the following way. They prepared filter papers with measured quantities of ether solutions with known acenaphthene content. After the evaporation of the ether, wheat grains were put for germination in the filter papers. By means of this method the threshold value of c-mitotic action was determined at 0,3 mg acenaphthene on 40 grains of wheat. To make direct comparisons with colchicine possible, and also to test our water-lipoid partition hypothesis, we had to apply the substances as water solutions.

Plant physiologists and others have for a long time used a method of preparing water solutions of plant hormones and other substances of low solubility. They make a strong alcoholic solution of the substance and then add a small quantity of this solution to a large quantity of water. PATTON and NEBEL (1940) used this procedure in preparing colloidal solutions of different hydrocarbons (including acenaphthene) in a study of their physiological and cytological effects. Their work, however, was not directed to the study of the c-mitosis, this effect of colchicine and acenaphthene being mentioned only very briefly (p. 612). EHRENBERG and ÖSTERGREN (1942) used similarly produced water solutions of acenaphthene in the study of the action of acenaphthene on the spindle apparatus of *Salix*, although the method used on that occasion was not described.

We prepared a series of solutions in absolute ethyl alcohol of each of the different substances which we wished to test. The concentrations of these alcoholic solutions were selected so that not more than 5 cc had to be added to 1 l. water (tap water being used throughout this investigation) in order to get the required concentrations. If less than 5 cc were added, the difference was added in the form of pure alcohol. Thus, all the solutions used contained 0,5 volume % of alcohol. In order to get the substance as efficiently dispersed as possible the alcoholic solutions were blown out through a pipette into the midst of the water, while this was kept in vigorous motion. In the cases when a precipitate was obtained, this was always very finely dispersed. Therefore it might be expected that any possibly occurring deficit of dissolved substance in the solution would rapidly be compensated.

Only very little is known about the solubility of the substances tested in this investigation (cf. the tables of SEIDELL, and of LANDOLT, BÖRNSTEIN, ROTH, and SCHEEL). The method employed by us for the preparation of the solutions yields, however, some information in this respect. If a remaining precipitate or opalescence arises when the alcoholic solution is mixed into the water, the solubility limit of the substance has been exceeded. It is true that the values which we obtain of the solubility of the substances are not the solubilities in pure water but in a 0,5 % solution of ethyl alcohol; however, the difference may well be negligible.

The solubility value in water of naphthalene obtained by us is in good agreement with that determined by MITCHELL (1926) by the aid of the refraction index. Our value for acenaphthene, on the other hand, is immensely smaller than that obtained by SHMUCK (1938). It seems but little probable that the solubility can be thus changed by the presence of 0,5 % alcohol. SHMUCK gives no particulars of the method according to which his value was determined.

It should be pointed out that the accuracy of the method used by us decreases rapidly, as the water solubility of the substance diminishes. If the solubility limit is exceeded by e. g. 10 %, a much larger amount of precipitate will arise if the substance is easily dissolved than if it is very slightly soluble.

Besides colchicine, the following substances were tested: naphthalene, the α-forms of naphthylamine, methyl-, chloro-, bromo-, and iodonaphthalene, the β-forms of methyl-, chloro-, and bromonaphthalene and acenaphthene. In the first experimental series (naphthalene, α-chloronaphthalene, acenaphthene) the following percentage

concentrations were used: 0,0001, 0,0005, 0,001, 0,002, 0,004 %. After that, the advantage of using molar concentrations was recognized; so the next series (α-naphthylamine, α-methyl-, α-bromo-, α-iodonaphthalene) included the following concentrations: 2, 4, 8, 16, 24, 32, 40, 80, 160, 240, 320, 400, 800, 1600, 2400 and 3200×10^{-6} mol per litre. The first two series were later on supplemented with some more determinations in molar concentrations.

In our experiments a South-European commercial variety of a large *Allium Cepa* was used, which was found to be very suitable for our purpose. It very rapidly formed a large number of roots, which allowed of each bulb being fixed several times. Its chromosome conditions were very clear and the least c-mitotic tendency was readily noticed. The onions were placed on pure tap water for one or two days. After that time the root tips were usually 5—10 mm in length, and the bulbs were then placed on glass jars containing the different solutions. The quantity of solution used in each case was 175 cc. Twelve hours before each fixing the roots were rinsed in water and the solution was renewed. This was done as a measure of precaution against putrefaction. Root tips were fixed after 12, 36 and 84 hours of treatment. Navashin was used as fixing fluid.

In one series *Pisum sativum* was treated instead of *Allium*. This experiment was performed in the same manner as described above for *Allium*. Each glass jar was covered by a piece of canvas, through which the roots of some 10 peas dipped into the fluid.

2. EXPERIMENTS WITH ALLIUM.

A. THE EFFECT OF COLCHICINE AND OF α-SUBSTITUTED NAPHTHALENE DERIVATIVES.

In this paragraph are also included acenaphthene, in which 2 α-positions are substituted by $—CH_2—CH_2—$, and naphthalene itself in which the α-position is occupied by —H. The results are summarized in Table 1. For each substance the following columns are given: (1) The concentration of the solution. Where percentage concentrations have been employed, they are converted in the table into molar concentrations. (2) The presence of a precipitate is indicated by a + in this column. (3) + in this column indicates the presence of the macroscopic colchicine effect, the c-tumour reaction. (4) + means the exclusive occurrence of c-mitoses in the studied slides, — means only normal mitoses, ± a transitional condition, both normal mitoses and

disturbed mitoses are present, $\underset{(-)}{+}$ = preponderantly c-mitoses and $\overset{(+)}{-}$ = preponderantly normal mitoses. (5) * = strong poison effect, root tips are quite slack, + = an evident poison effect on the mitoses, — = no perceivable poison effect.

In the table the substances are arranged in order of solubility. As seen, the solubility of colchicine is of quite another order than that of the naphthalene series. It is given by MULLER (1903, cited from SEIDELL) as 9,6 g per 100 g water. We already got traces of a precipitate in 3 % (75180×10^{-6} mol). Probably this precipitate was due to the presence of an impurity; it did not seem to increase with an amount corresponding to the added colchicine when the colchicine concentration was increased from 3 to 8 %. Distilled water was used in these high colchicine concentrations. [LEVAN and SANDWALL (1943) found a rich crystalline precipitate in 4 % colchicine solution *in wort*]. Among the naphthalene series the seven substances tested form the following sequence in order from higher to lower solubility: naphthylamine, naphthalene, methylnaphthalene, chloro-, bromo-, iodonaphthalene, acenaphthene. The limits of the lowest concentration with a precipitate or an opalescence range from 400×10^{-6} mol in naphthylamine to 24×10^{-6} mol in acenaphthene.

The c-tumour reaction is characteristic both of c-mitotic substances and of phytohormones (LEVAN, 1939). It is caused by a swelling of the cortical cells, lying immediately above the meristem. This reaction usually runs parallel with the c-mitotic action. In the present experiments, however, it shows a certain autonomy. Thus, c-mitosis is found in certain weak concentrations of acenaphthene and bromonaphthalene, in spite of the absence of c-tumours. Further, it turned out that c-tumours were not found in the strongest concentrations of colchicine, naphthylamine and chloronaphthalene, some of which had clear c-mitoses. The poison effect, which inhibits growth, evidently also checks the production of c-tumours.

Column 4 shows the c-mitotic effect. It is seen that all the tested substances show c-mitosis in some concentrations. The course of the c-mitosis was typical, and the occurrence of c-mitosis within the tissue was often very complete. In the concentrations above 6×10^{-6} mol of acenaphthene, for instance, no difference at all could be found as compared with colchicine-induced c-mitosis. There were always found, however, in the vicinity of the threshold value a number of concentrations in which c-mitoses occurred intermingled with normal mitoses. Thus, the starting of the c-mitosis could take place typically, although

TABLE 1. *Colchicine, acenaphthene and the α-naphthalene series.*

Colchicine without alcohol					α-naphthyl-amine					Naphthalene					α-methyl naphthalene					α-chloro-naphthalene					α-bromo-naphthalene					α-iodo-naphthalene					Acenaphthene				
Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect	Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect	Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect	Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect	Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect	Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect	Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect	Concentration in 10^{-6} mol	Precipitate	c-tumour	c-mitosis	Poison effect
200480	?	—		*																																	
75180	?	—	+	+																																	
25060	—	+	+	—																																	
					3200	+	—		*																													
2506	—	+	+	—	2400	+	—		*																													
1253	—	+	+	—	1600	+	—		*																													
					800	+	—	+	*																													
					400	+	—	±	+						400	+	—	+	*																				
					320	—	—	(+)	+	312	+	+	+	+																									
251	—	+	+	—	240	—	—	—	+						240	+	—	+	*	246	+	—	+	*	240	+	+	+	*	240	+	+	+	+	260	+	+	+	—
200	—	+	±	—	160	—	—	—	+	156	—	+	+	—	160	+	+	+	+						160	+	+	+	*	160	+	+	+	—	130	+	+	+	—
150	—	+	(+)	—											120	—				123	+	—	+	*															
100	—	—	—	—	80	—	—	—	—	78	—	±	+	—	80	-	+	+	—	80	—				80	+	+	+	+	80	+	+	+	—					
																				62	—	+	+	—	60	+								65	+	+	+	—
50	—	—	—	—	40	—	—	—	—	39	—	—	—	—	40	—	+	±	—						40	—	+	+	—	40	+	+	+	—					
					32	—	—	—	—						32	—	+	±	—	31	—	+	+	—	32	—	+	+	—	32	+	+	+	—	32	+	+	+	—
					24	—	—	—	—						24	—	—	(+)	—	20	—	+	+	—	24	—	±	+	—	24	—	+	+	—	24	+			
					16	—	—	—	—						16	—	—	(+)	—	10	—	(+)	±	—	16	—	—	±	—	16	—	+	+	—	16	—			
					8	—	—	—	—						10	—	—	—	—	8	—	—	—	—	8	—	—	±	—	8	—	±	(+)	—					
										7	—	—	—	—						7	—	—	—	—	7	—	—	±	—									
																				6	—	—	—	—	6	—	—	±	—						6	—	—	+	—
																									5	—	—	—	—						5	—	—	(+)	—
					4	—	—	—	—																4	—	—	—	—	4	—	+	—	—	4	—	—	—	—
					2	—	—	—	—																2	—	—	—	—	2	—	—	—	—	1	—	—	—	—
																																		0,6	—	—	—	—

the spindle mechanism did not need to be completely anesthetized and multipolar anaphases attended by micronuclei and microcytes were found. 800×10^{-6} mol naphthylamine acting for 12 hours, for instance, had that effect.

α-iodonaphthalene behaved somewhat differently from the other substances. The c-mitotic effect of this substance became complete earlier in the middle of the concentration range than in the strongest solutions. Even the saturated solutions 32—40 $\times 10^{-6}$ mol showed after 12 and 36 hours a mixture of normal mitosis and c-mitosis, while the concentrations 16 and 24×10^{-6} mol after the same times had almost exclusively c-mitoses. After 84 hours, however, the c-mitotic effect had become complete within the entire concentration range from 16×10^{-6} mol and upwards. Traces of c-mitosis could be seen as far down as in 4×10^{-6} mol.

It is very evident from the table that the threshold value of c-mitosis sinks parallel to the water solubility. In α-naphthylamine, which has a high water solubility, typical c-mitoses are found only in the strongest concentrations in which the poison effect allows any divisions to occur. Full c-effect was obtained only in 800×10^{-6} mol, and this only on the first occasion of fixing (treatment for 12 hours). On the second occasion, all mitoses had already disappeared in the concentrations which had shown c-mitosis, and after 84 hours all roots in the critical concentrations were dead.

It is quite a characteristic feature that the occurrence of c-mitosis can be observed only during the first day of treatment in those substances in which any considerable poison effect is present. This was found to be the case, for instance, in saturated solutions of chloronaphthalene.

Naphthalene, which follows naphthylamine in solubility, has the highest threshold value among the remaining substances; thereafter come methylnaphthalene, chloronaphthalene, and so on. Iodonaphthalene, which has the lowest solubility among the monosubstituted α-derivatives, also has the lowest threshold value. It still shows traces of c-mitosis in 4×10^{-6} mol. Acenaphthene, however, has a higher position of its threshold than iodonaphthalene, in spite of its solubility being lower. As acenaphthene has two α-positions substituted it is very questionable, however, whether it may be considered to belong to the same series as the other naphthalene derivatives treated in this paragraph. The threshold concentration of acenaphthene (5×10^{-6} mol) is 30 times lower than that of colchicine. When colchicine, too, is

applied in a 0,5 % alcohol solution this number is changed to 80 times (see below).

Thus, it can be said already now about the two problems which we desired to elucidate by these experiments that, firstly, *acenaphthene and all the other tested naphthalene substances with the exception of naphthylamine are active in lower concentrations than colchicine.* The superiority of colchicine as a polyploidizing agent is therefore due to the great practical advantages of its water solubility and to the absence of poison effect within a wide concentration range. This question will be more closely discussed in a later chapter. Secondly, it may be said that the experiences from the naphthalene series *strongly support our view of a connection between activity and water solubility*, a view suggested by the results of SHMUCK and GUSSEVA.

All the naphthalene derivatives studied, except acenaphthene, exhibit poison effects in the stronger concentrations (see column 5 of Table 1). The poison effect does not show connection with the water solubility so clearly as the c-mitotic effect did. Any connection which may be present seems to be of quite another nature. The poison effect increases with increasing water solubility. It seems as though the concentration of the substance has to exceed about $160—240 \times 10^{-6}$ mol if a poison effect is to originate. Owing to this condition it does not show up in the substances with the lowest solubility.

Strangely enough, the observation was made that saturated solutions of one and the same substance might have a poison effect of varying strength, depending on the quantity of undissolved substance present. Their effects would of course have been expected to be identical, since they ought to contain the same concentration of the substance in solution. A similar difference in the effect of saturated solutions was shown very strikingly by LEVAN and SANDWALL (1943) by treating yeast with camphor and borneol. Different explanations of this phenomenon may be suggested. Possibly the living cells have the power to destroy the substances in question at their metabolic processes. The deficit of the substances thus originating would be more rapidly compensated if a larger quantity of precipitate were present.

A second possibility is that the effect is due to the presence of traces of impurities. A solution that is saturated as far as the main substance is concerned need not, of course, be saturated in respect of possible impurities. So the concentration of the impurities varies within the range of saturated solutions.

Still a third possibility may be suggested. The cells may be able to absorb not only dissolved substance but also substance suspended in the fluid. It is easy to understand that such a thing might happen in the case of naked animal cells. It is harder to understand in the case of plant cells, which are surrounded by a cellulose wall, soaked with water. The cell membrane, however, also contains a certain quantity of waxes, which are already present in the primary membrane (FREY-WYSSLING, 1938 a, p. 225). If a small particle or droplet of the substances sticks to the root, it is therefore very plausible that the substance may penetrate into the cell owing to the wax content of the cell wall, without first having to pass through water. This mode of penetration may facilitate a more rapid storage of the substance in the cell, and, if the substance is broken down by the cell, actually means a higher concentration of the substance in the cell. Evidence that substances of the nature of lipoids may pass through cell walls is afforded by the well-known excretion of waxes on many leaves.

B. COMPARISON OF THE EFFECT OF VAPOUR AND SOLUTION OF ACENAPHTHENE.

In order to compare the effects of vapours and water solutions of acenaphthene, some bulbs were placed on glass jars, the insides of which were covered with moist filter paper in which crystals of acenaphthene were scattered. This experiment was started at the same time as bulbs were put on the acenaphthene solutions, as described in the preceding paragraph.

A decided difference in response could be recorded between the bulbs treated with vapour and those with saturated solution. The c-tumours developed much more rapidly and grew stouter under the treatment with vapour. On the other hand, the development of c-mitoses occurred more completely and with greater regularity in the solutions. Thus, the saturated solution already gave complete c-mitosis in 12 hours, while the treatment with vapour only yielded transitory forms between normal and c-mitosis in the same period. In the later fixations even these disturbances had recovered, almost exclusively normal mitosis being found. At the treatment with solutions, on the other hand, the c-mitosis prevailed at the later fixations as well.

Thus, the treatment with vapour seems to be superior in provoking tumour formation, while the treatment with dissolved substance is more apt to induce c-mitosis. This gives additional support to the

view that these two reactions, the c-tumour formation and the c-mitosis, are relatively independent of each other.

Of interest for the understanding of the difference in action between vapour and solution is no doubt the dissimilarity in action on the hormone distribution of plants between moist air and water described by BORGSTRÖM (1939 c, pp. 36—38 and 136—137). The formation of root hairs, which may be stimulated by growth substances (BORGSTRÖM, 1939 b), is also promoted by moist air. Since the growth substances are able to give rise to a similar tumour formation as the c-mitotic substances (LEVAN, 1939), and since moreover they are known (DUHAMET, 1939) to act antagonistically to the growth-checking effect of colchicine (probably c-mitosis), it is not so difficult to understand that moist air may promote the formation of c-tumours and at the same time check the c-mitotic effect.

C. THE ANTAGONISM OF ETHYL ALCOHOL TO COLCHICINE.

With a view to compare the effect of naphthalene derivatives and of colchicine, we arranged parallelly with the first experimental series one series with different colchicine concentrations, all of which contained 0,5 vol. % ethyl alcohol, as did all the solutions of the naphthalene series. We were somewhat astonished to find that the threshold value of colchicine turned out to be situated decidedly higher than had been earlier determined by the senior author (LEVAN, 1938), who then found that the lowest active concentration was 0,0055 %. In the presence of 0,5 % alcohol even 0,008 % was without any effect.

In order to test the phenomenon indicated by these results two experimental series were arranged (Table 2): in one of them a number of colchicine concentrations in pure water were compared with the same concentrations in 0,5 % alcohol, in the other they were compared with a series containing 2 vol. % alcohol. As seen from Table 2, an addition of 0,5 % alcohol increases the threshold value of the c-tumour reaction from 150×10^{-6} mol (0,006 %) to 200×10^{-6} mol (0,008 %) and that of the c-mitosis reaction from 150×10^{-6} mol (0,006 %) to 250×10^{-6} mol (0,01 %). An addition of 2 % alcohol has a still more pronounced action, only few indications of c-tumours and c-mitoses being found in 250×10^{-6} mol, while most cell divisions take place quite normally with normal bipolar spindles. The 2 % alcohol, however, brought about a very decided poison effect, with the result that certain fixations only contained very few mitoses.

The experiment proves very clearly that alcohol exercises an an-

tagonistic action on the colchicine effect. In fact, alcohol acts as an antidote to colchicine. As already mentioned in the preceding paragraph, other substances are known to have such an effect. DUHAMET's (1939) report of this effect of heteroauxin has already been cited. HAVAS (1940) also observed such an antagonistic effect of heteroauxin in plants and even in animals. He also observed (1938, 1940) an antagonism in some respects between ascorbic acid and colchicine. TÖRÖ (1938) and TÖRÖ and VADÁSZ (1939) describe an antagonistic action to colchicine of a substance obtained from embryonic heart tissue, named corhormone. This substance, however, was specific in

TABLE 2. *The antagonism between alcohol and colchicine.*

Concentration in 10^{-6} mol	Colchicine without alcohol						Colchicine with 0,5 % alcohol						Colchicine without alcohol				Colchicine with 2 % alcohol			
	24 hours		48 hours		96 hours		24 hours		48 hours		96 hours		30 hours		72 hours		30 hours		72 hours	
	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis	c-tumour	c-mitosis
2500	+	+	+	+	+	+	+	+	+	+	+	+								
1250	+	+	+	+	+	+	+	+	+	+	+	+								
250	+	±	+	±	+	+	+	±	+	±	+	+	+	+	+	+	(+)	(+)	(+)	—
200	±	—	+	±	+	±	±	—	+	—	+	—	+	±	+	+	—	—	—	—
150	(+)	—	+	(+)	+	(+)	—	—	—	—	—	—	+	±	+	±	—	—	—	—
100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	—	—	—

its action. It was active only within the heart muscles and not in other tissues. Cells treated exclusively with corhormone exhibited an immense increase in the mitotic rate (400 %). Especially the velocity of the metaphase stage was speeded up. Colchicine, on the other hand, exercised a retarding action on the divisions, especially the metaphase having a tendency to being checked. A direct ability to abolish the effect of colchicine was also shown in the corhormone.

Certain reports that ethyl alcohol in some concentrations may stimulate the mitotic rate are of great interest in this connection. MALTAUX and MASSART (1906, cited from TISCHLER, 1942, p. 59) recorded the time of the mitosis of *Chilomonas Paramaecium* at 0 % alcohol as 33 minutes, but at 6—7 % alcohol as 15 minutes.

The occurrence of substances with an effect antagonistic to colchicine makes it seem possible that the differences in sensitivity to colchicine which have been established between different groups of organisms may be due, at least in part, to the presence of such substances. It may be rather suggestive that yeast, which is strikingly insensitive to colchicine (RICHARDS, 1938; LEVAN and SANDWALL, 1943), is characterized by its abundant production of alcohol during its metabolic processes.

D. THE EFFECT OF β-NAPHTHALENE DERIVATIVES.

According to SHMUCK and GUSSEVA (1939, 1940 a, b and c) all the studied β-substituted derivatives of naphthalene were inactive. A c-mitotic action of β-substituted naphthalene ethers has nevertheless been recorded by GAVAUDAN, GAVAUDAN, and DURAND (1939 a). This effect, however, is denied by SHMUCK and GUSSEVA (1940 a), who presume that the effect was due to the presence of impurities such as, for instance, traces of the corresponding α-derivatives. As our method of treatment has been very effective in revealing any occurrence of a c-mitotic activity, we considered it worth while also to test a few β-derivatives of naphthalene.

β-methyl-, β-chloro- and β-bromonaphthalene were chosen for this test. We found the β-forms to be of somewhat lower solubility than the corresponding α-forms. The lowest concentration with a precipitate was the following:

β-methylnaphthalene: ..	80×10^{-6},	the α-form:	160×10^{-6}
β-chloronaphthalene: ..	80×10^{-6},	» α »	123×10^{-6}
β-bromonaphthalene: ..	40×10^{-6} (or lower),	» α- »	60×10^{-6}

The formation of c-tumours occurred very clearly in the β-chloro- and β-bromo-series, in which only the weakest concentration of β-chloronaphthalene was devoid of c-tumours. The tumour formation was less prominent in the β-methylnaphthalene series, only one concentration (160×10^{-6}) showing tendency to c-tumours.

The microscopical examination immediately showed that the β-forms were less efficient in inducing c-mitosis than the α-forms. The α-forms provoked complete c-mitosis all through the concentration range in question. Among the β-forms only β-methylnaphthalene gave complete c-mitosis and this effect was obtained only in the two strongest concentrations. The other two substances gave only a mixture of c-mitoses and normal mitoses. Here, too, in the β-forms a

certain independency may be noticed between the c-tumour reaction and the c-mitosis reaction.

It might perhaps be suggested that a small intermingling with the α-form is present and causes the effect. Especially in chloronaphthalene, however, where a mixture of normal mitoses and c-mitoses occurs from 40 to 320 $\times$ 10^{-6} mol, this explanation is less probable. With such an increase in concentration of the impurity full c-mitosis should prevail in the stronger concentrations. It should also be noticed that the β-chloronaphthalene used was of a high degree of purity (the quality denoted by »reinst» from TH. SCHUCHARDT, Görlitz, Germany).

TABLE 3. *The β-naphthalene series.*

Concentration in 10^{-6} mol	β-methyl-naphthalene				β-chloro-naphthalene				β-bromo-naphthalene			
	Precipitate	c-tumour	c-mitosis	Poison effect	Precipitate	c-tumour	c-mitosis	Poison effect	Precipitate	c-tumour	c-mitosis	Poison effect
320					+	+	±	*	+	+	$\frac{(+)}{-}$	+
240	+	−	+	*	+	+	±	+	+	+	$\frac{(+)}{-}$	−
160	+	±	+	+	+	+	±	−	+	+	$\frac{(+)}{-}$	−
80	+	−	±	+	+	+	±	−	+	+	$\frac{(+)}{-}$	−
40	−	−	$\frac{(+)}{-}$	−	−	−	±	−	+	±	−	−

Thus, it must be said that there is good reason to ascribe a tendency of inducing c-mitosis also to the β-derivatives of naphthalene. *The fundamental difference between the α-and β-derivatives consists according to our opinion in the threshold value of the c-effect being closer to the point of saturated solution in the β-forms, the range of active concentrations therefore being much smaller.* The failure of some earlier workers to induce c-mitoses by means of the β-forms is probably due to the fact that by their methods a sufficiently high concentration of the substances was never attained within the cells. Another factor of importance may be that the earlier treatment were always made in moist air instead of water solution. And, as was discussed in paragraph B of this chapter, moist air seems to have a tendency to counteract the origin of c-mitoses.

3. EXPERIMENTS WITH PISUM.

In one of his first communications on the effect of acenaphthene KOSTOFF (1938 b) reports that Leguminosae need longer treatments than Gramineae for responding with c-mitoses. Several other workers have made similar observations that entire plant groups differ in their sensitivity to certain of the c-mitotic substances. SHMUCK and GUSSEVA (1939) tested peas, vetch and clover by germinating them on filter papers containing acenaphthene, α-chloro-, bromo- and iodo-naphthalene as well as several other derivatives of naphthalene. Their result was negative in all instances except in α-nitronaphthalene and α-ethylnaphthoate. LEVAN (unpublished) tested species of *Caragana*, *Cytisus*, *Dolichos*, *Galega*, *Glycine*, *Laburnum*, *Lens*, *Lotus*, *Melilotus*, *Onobrychis*, *Ornithopus*, *Phaseolus*, *Pisum*, *Robinia*, *Thermopsis*, *Trifolium*, *Trigonella*, and *Vigna* with vapour of α-chloronaphthalene. Stem buds of *Melilotus albus* were the only instance in which any c-mitotic reaction was obtained. WEICHSEL (1940) and JARETZKY and SCHENK (1940) also report negative results from their experiments of treating leguminous plants with acenaphthene.

It was, however, not only the results of KOSTOFF which made it evident that the immunity of Leguminosae to naphthalene derivatives could not be absolute. Thus, ONO (1940) succeeded in producing a tetraploid *Pisum sativum* by treating germinating seeds with acenaphthene. In order to investigate this question we studied the reaction of germinating peas to colchicine, naphthalene, α-chloronaphthalene and acenaphthene. Table 4 gives a summary of the results.

A striking feature in this series is the total absence of c-tumours in all concentrations of the naphthalene substances. C-tumours are found only in the colchicine series. But the growth in length is also considerably checked by the naphthalene substances, as is seen from the last column in each series, which gives the mean length of five roots after a treatment lasting three days. Their original length was ± 30 mm.

The threshold values of c-mitosis have quite a different position in *Pisum* than in *Allium*. In the case of colchicine the threshold value is situated lower than in *Allium*. Full c-mitosis was obtained in 75×10^{-6} mol (= 0,003 %) while *Allium* (see Table 1) gave full c-mitosis first in $200—250 \times 10^{-6}$ mol (0,038—0,01 %). In all the naphthalene derivatives, on the other hand, the threshold values are moved upwards. Still, all the three substances give c-mitosis even if the effect is less uniform and more casual than in *Allium*. In spite of the fact that all the tested naphthalene derivatives induced c-mitosis at least

TABLE 4. *Experiments with Pisum.*

Colchicine	Concentration in 10^{-6} mol	251	75	25	8	
	Precipitate	–	–	–	–	
	c-tumour	+	+	–	–	
	c-mitosis	+	+	–	–	
	Poison effect	–	–	–	–	
	Length of roots after 84 hours	48,8	43,2	69,4	71,0	
Naphthalene	Concentration in 10^{-6} mol	312	156	78	39	7
	Precipitate	+	–	–	–	–
	c-tumour	–	–	–	–	–
	c-mitosis	(+)	–	–	–	–
	Poison effect	–	–	–	–	–
	Length of roots after 84 hours	50,3	50,7	64,4	57,2	68,0
Chloronaphthalene	Concentration in 10^{-6} mol	246	123	62	31	6
	Precipitate	+	+	–	–	–
	c-tumour	–	–	–	–	–
	c-mitosis	0	0	±	(+)	–
	Poison effect	*	*	–	–	–
	Length of roots after 84 hours	41,4	44,8	44,6	47,8	62,8
Acenaphthene	Concentration in 10^{-6} mol	260	130	65	32	6
	Precipitate	+	+	+	–	–
	c-tumour	–	–	–	–	–
	c-mitosis	+	±	±	(+)	–
	Poison effect	±	–	–	–	–
	Length of roots after 84 hours	33,8	43,0	42,2	43,7	58,0

in some degree, no traces of c-tumours were found. In *Pisum*, therefore, the c-tumour reaction seems to be entirely separated from the induction of c-mitoses.

The sequence of activity of the different substances is probably the same here as in *Allium*. Naphthalene here, too, is the least active of the tested substances. It has a weak c-mitotic effect only in the strongest solutions (312×10^{-6} mol and 156×10^{-6} mol). The observations do not make it possible to decide with certainty whether acenaphthene is more effective than α-chloronaphthalene. Acenaphthene, however, is the only substance which in any concentration (260×10^{-6} mol) gives full c-mitosis, but the poison effect conceals the result in the strongest concentrations of α-chloronaphthalene.

Thus, it must be regarded as proved that most of the failures to induce c-mitoses in Leguminosae by means of naphthalene derivatives have been due less to any absolute inactivity of the substances than to the technique used. The difficulty of obtaining a sufficiently high concentration within the tissue may have played a part, together with the counteracting of the c-mitotic effect consequent upon the treatments having been made in moist air instead of in water.

II. THEORETICAL PART.

1. THE PRACTICAL APPLICABILITY OF C-MITOTIC SUBSTANCES.

A detailed examination on a quantitative basis of the qualities of the c-mitotic substances, a first attempt at which is made in the present study, must necessarily be of great importance for the understanding of the practical use of these substances. A factor that is undoubtedly of very great significance from a practical point of view is the presence or absence of a poison effect.

On the other hand, it seems to be of less consequence whether the substance is active in a high or a low concentration. For, if the substance acts only in a high concentration, this difficulty should be easily removed by using a stronger solution. Even if the lower threshold value for c-mitotic effect may be used as a measure of the immanent activity of the substance in influencing mitoses, this value has little significance as a measure of the practical applicability. This is clearly seen from a comparison between colchicine and the naphthalene derivatives.

The practical applicability is no doubt dependent on a great many

various factors, most of which are perhaps not approachable for an exact study. One important factor is, according to our opinion, the size of the active concentration range. The decisive moment is of course not so much the absolute size of this range as its *relative size*, i. e. the relation between its upper and lower limits.

It may be considered as rather evident that at the treatment of a plant tissue the concentration within the tissue will be lower than in the surrounding solution. The penetration of the substance is associated with some difficulty. It may be supposed for the sake of simplicity that at its penetration through the tissue the substance encounters the same resistance at equal distances. The concentration may, for instance, be reduced by one-half for each millimetre. This connection may be expressed mathematically:

$$C = C_0 K^{-D},$$

where C_0 = the concentration outside the tissue and C = the concentration at the distance D in the tissue. This equation may be changed into:

$$D = \frac{\log C_0 - \log C}{\log K}.$$

The greatest value which D can assume with maintained c-mitosis is obtained if C_0 is made equal to the solubility S_w and C = the threshold concentration T_w.

$$D_{max} = \frac{\log S_w - \log T_w}{\log K} = \text{const.} \times \log \frac{S_w}{T_w}$$

Thus, it is evident that the effective permeability of a substance is a logarithmic function of the relation between the limits of its active concentration range. The wider this active range is, the greater is the assurance that a sufficiently high concentration will be obtained within the tissue, and the larger will be the part of the tissue attainable for the action.

In Fig. 1 this factor is graphically represented on the basis of our results in *Allium*, viz. the logarithm of the relation between the limits of the active concentration range. The upper limit is the saturated solution, the lower limit the threshold value of action. The zone in which a poison effect may be supposed to be present is drawn in outline, the rest is blacked in. The poison zone must of course be subtracted when an estimate is being made of the practical applicability.

In some cases a distinct poison effect was obtained only in solutions containing a considerable excess of precipitate. We do not consider it likely that the concentration in such a case is higher than in a really saturated solution. This peculiar situation is more probably due to a more rapid supply of consumed substance. We have therefore marked in the diagram the poison limit slightly below the concentration of saturated solution. A weak point in this, however, may be the previously mentioned possibility of the tissue absorbing not only dissolved substance but also substance present in suspension. We do not believe,

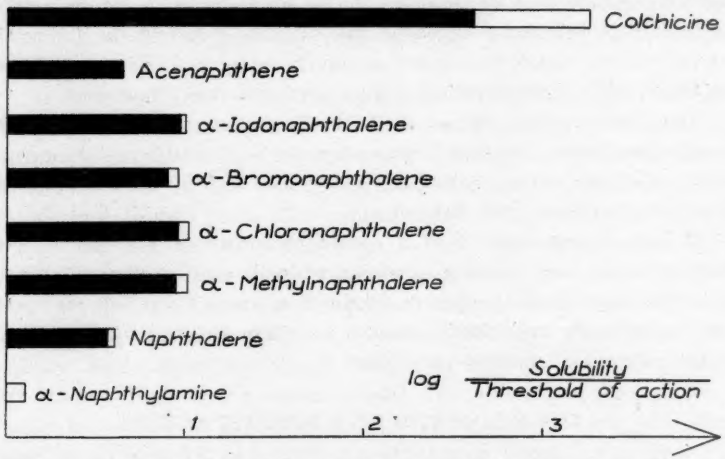

Fig. 1. The relative size of the active concentration ranges of the different substances.

however, that this possibility changes the situation to any considerable extent.

The poison zone as well as the other features of the diagram are of course indicated very approximatively. The small number of concentrations in each series within the critical ranges prevents a more exact construction of the diagram. But in order just to illustrate our principle and to give an approximate idea of the differences present a greater accuracy is not needed. The solubility of colchicine used in the diagram is that given by MULLER (cf. SEIDELL).

The great superiority of colchicine as a polyploidizing agent is clearly demonstrated in the diagram. An especially valuable feature of acenaphthene is its lack of toxic properties, as is also stressed by KOSTOFF (1938 a). Acenaphthene is also the substance which, next to

colchicine, is responsible for the largest number of the chemically induced polyploids. The α-halogen-naphthalenes and the α-methyl-naphthalene are poisonous in their strongest concentrations. As they, nevertheless, have a relatively large range, without toxic properties, they might have some practical value. No polyploids are reported, however, from treatment with these substances. This may be due to the fact that they have not been extensively tried in polyploidization experiments. α-naphthylamine is completely useless, because of the strong poison effect present all over the active range. It is probable that chloral hydrate is a substance that behaves similarly to α-naphthylamine. The essential difference between the α- and β-derivatives of naphthalene, which makes the latter so much more inactive, is probably that their active concentration ranges are immensely narrower.

The value of the constant in the last equation will vary from substance to substance. Its size is dependent on the variable resistance met by the substance in penetrating the tissue, and also by the power of the living cells to destroy the substance.

It is not impossible that a continued study of substances with c-mitotic action may reveal a substance which is as efficient as colchicine, and at the same time cheaper in production. Also from this viewpoint it is consequently important to make a systematic investigation of the c-mitotic action of various substances.

2. THE MECHANISM OF C-MITOTIC ACTION.

A. DIFFERENT EARLIER OPINIONS.

The significance of *chemical structure* for the activity of the c-mitotic substances has been examined by several authors. SHMUCK (1938) assumed a chemical structure similar to that of the carcinogenic hydrocarbons. SHMUCK and GUSSEVA (1939, 1940 c) made further studies from this viewpoint. They found, as already mentioned, that if naphthalene or acenaphthene was substituted by certain radicals known to extinguish the activity of carcinogenic hydrocarbons, the c-mitotic activity was suppressed. They also established that the position of the substituents is of great significance. This is the case, for instance, with the α- and β-position in the naphthalene derivatives (1939, 1940 a, b, and c). FAVORSKY (1939) stressed the importance of the amino-group. Also the nitro-group and halogenes may increase the activity. C-mitotic activity is not limited to the cyclic substances, purely aliphatic compounds may also be active, as, for instance, ether and chloral hydrate.

FAVORSKY's somewhat pessimistic conclusion was: »The organism is endowed with a constant response to widely varying stimulation» (l. c., p. 74). SIMONET and GUINOCHET (1939 b) emphasize the significance of the aromatic nucleus, and the significance of suitable substituents, which reduce the poisonous effect and promote »les propriétés mito-inhibitrices».

Several workers have made comparisons between colchicine and different chemically related substances (BRUES and COHEN, 1936; LUDFORD, 1936; BHADURI, 1939). LETTRÉ, ALBRECHT and FERNHOLZ (1941) and LETTRÉ (1942) made a systematic investigation to determine what radicals of the colchicine molecule were necessary for its characteristic action.

A study by WADA (1940) lays special stress upon a *physical property* of colchicine. He assumes that the action of colchicine is mainly due to its property of being a substance with surface activity, reducing the surface tension of the »Atraktoplasma». Some changes of a more »qualitative» nature were also supposed. SHIGENAGA (1937), studying spindle disturbances in living cells induced by various poisons, including chloral hydrate, drew the conclusion that they all acted in the same manner as hypertonic sugar solutions, viz. by causing a dehydration of the »kinoplasm». However, the fact that both chloral hydrate and hypertonic sugar solutions may bring about mitotic disturbances that show a certain superficial similarity to each other can hardly be considered sufficient proof that their mechanism of action is identical. From BĚLAŘ's (1929 a and b) very detailed and very clear descriptions of the action of hypertonic solutions on plant and animal mitoses it is evident that the disturbances, induced in that manner, are of quite another nature than the c-mitotic disturbances.

GAVAUDAN and GAVAUDAN (1940) made an attempt to correlate the activity of the c-mitotic substances with their physical and chemical properties. They made comparisons of the boiling points, melting points and solubilities of these substances. The examination gave the impression that a decreasing solubility was attended, firstly, by a reduced poison effect and, later on, also by a reduced c-mitotic activity. These authors point out that conclusions can be drawn only from closely related substances. Thus, the differences between the α- and β-derivatives of naphthalene may depend on the difference in melting point and solubility. The β-derivatives have a considerably higher melting point and lower solubility, and that might be the cause of their lower activity. SIMONET and GUINOCHET (1939 a) observed, on comparing the effect on

wheat and flax of different substances, that flax, which was the more resistant material, could be induced to full c-mitosis only by colchicine, which was the most soluble substance of those tested. The reason why we arrived at quite different results lies in the fact that we made quantitative determinations of the threshold value of activity instead of only trying to estimate the degree of disturbances after treatment with vapour.

RIES (1939) concluded that colchicine »*in erster Linie auf die Chromatinstrukturen wirkt*» (italics by RIES). He observed in *Periplaneta* a coarsening of the chromatin structures of resting nuclei »jedenfalls auf extreme Dosen». This opinion may be influenced by the DUSTIN-school, the members of which presume that colchicine stimulates the cells to mitosis (an opinion shared by RIES). They count colchicine among the so-called »poisons caryoclasiques», which are considered to have a specific action on the nuclei. According to our opinion the observation made by RIES on the resting nuclei is a trivial poison effect which has nothing to do with the specific properties of colchicine. It may be something analogous, although less extreme, to the changes of the resting nuclei noticed by GARRIGUES (1939) after treatment with chloral hydrate. It may be considered as established that colchicine does not stimulate mitosis. This impression of a stimulation is given by the accumulation of arrested metaphases in the tissue (LUDFORD, 1936; BRUES, 1936; CLEARKIN, 1937; GAVAUDAN and GAVAUDAN, 1938; MANGENOT, 1939, 1942; BUREAU and VILTER, 1939 a and b).

In our opinion the particular action of colchicine lies in its very specific effect on the spindle or on the mechanism underlying the formation of the spindle, while its action on the chromosomes is very slight. The supercontraction of the chromosomes often encountered in the c-metaphase is probably a secondary effect due to the prolonged metaphase stage. Even other influences which retard the mitotic process, for instance, cold, have a similar effect. MANGENOT (1942) points out that colchicine has no direct effect on the spiralisation of the chromosomes, its action being a specific paralyzing of the spindle.

It may also be mentioned, without entering into details, that many workers have examined or discussed the different *physiological changes* induced by c-mitotic substances, for instance, changes in osmotic pressure, p_H, action on enzymes, plasmatic viscosity, and so on (e. g. GUINOCHET, 1940; LEFÈVRE, 1939; BEAMS and KING, 1938; SHMUCK, GUSSEVA, and ILJIN, 1939; KING and BEAMS, 1940).

B. THE RELATIVE AUTONOMY OF THE C-TUMOUR MECHANISM.

The present investigation has shown that the tested substances have several different physiological activities. Thus, the following effects may be distinguished: (1) the general poison effect, (2) the c-mitotic action, (3) the induction of c-tumours. These different actions seem to be more or less independent of each other, and they have differently located threshold values. Whereas the c-mitosis and the c-tumour reaction often accompany each other and both show a distinct connection with the water solubility (threshold positively correlated to solubility), the poison effect is quite independent of these two effects. Its connection with the water solubility is of another nature than the c-mitosis and the c-tumour reaction: since the poison effect occurs most distinctly in stronger concentrations, it appears most evidently in the substances which are more easily soluble in water. There can hardly be any doubts that the biochemical mechanism of the poison effect is different from that of the c-mitosis and c-tumour effects. In the present paragraph a few opinions concerning the latter of these two effects will be discussed.

The first suggestion concerning the causes of c-tumour formation was that is was due to the increase in cell size brought about by the polyploidy (e. g. LEVAN, 1938; KOSTOFF, 1940 b). MANGENOT (1938) pointed out the connection of the tumefaction with the growth. Young roots, which grow rapidly, will get more pronounced tumours than old roots, which grow more slowly. Roots which are not elongating do not form any tumours. The swelling takes place in the extension zone, but if the treatment is prolonged for a long time the tumour will extend all over the tip region. The tumefaction is caused by each cell increasing in size, the number of cells remains unchanged. Thus, MANGENOT concludes that the tumour formation »est le résultat d'un trouble de la croissance de racines, trouble d'autant plus marquée que la croissance est plus active». MANGENOT (1939) is of opinion that because of the checking of the proliferation of the meristematic cells by the colchicine the cells of the extension zone will not be renewed. The cells located in this zone will therefore remain longer than usual in this position with its special conditions of supply of nourishment and growth substances. This is the cause of their abnormally strong growth. The submeristematic position of the tumefaction is also pointed out by GAVAUDAN, GAVAUDAN and POMRIASKINSKY-KOBOZIEFF (1937) and by O'MARA (1939).

HAVAS (1938) assumed an action of the colchicine on the growth

hormones, which were thought to cause the tumour formation. He had observed various hormone actions of colchicine and suggested the following possibilities as to their action: (1) colchicine might mobilize the hormones already present in the plants; (2) there might be brought about a deviation of the polarity of their transport, causing a more or less localized »hyper- or hypo-auxonomy»; (3) the endogenous phytohormones (»auto-auxones») may become inactivated locally or generally. Similar statements are also made on later occasions (1939 a and 1940). GAVAUDAN and GAVAUDAN (1939) likewise assume that the tumefaction might depend on a modification in the normal movement of the growth hormones.

Other growth disturbances by c-mitotic substances were found by KOSTOFF (1938 f), who noticed a swelling of the pollen tube under the influence of acenaphthene. This was considered to be due not exclusively to the increase in chromosome number but also to »simple cell expansion». (We think it questionable whether the number of chromosomes in a pollen tube may be considered to increase when the second pollen mitosis is rendered abortive. Anyhow, there is the same number of chromosomes present in the pollen tube after c-mitosis as after normal mitosis.) In this connection the characteristic camphor reaction of yeast may also be mentioned. This reaction involves a swelling of the yeast cells into large tube-like formations (BAUCH, 1942 c; LEVAN and SANDWALL, 1943). Camphor, too, as we have found, is a c-mitotic substance.

A strong support for the view that the c-tumours are not caused by the induced polyploidy of the cells is furnished by the observations of LEVAN (1942) that *Allium* bulbs which as a result of X-ray irradiation show no mitoses nevertheless develop typical c-tumours at the tension growth of the roots. Additional support for the view that the c-tumour reaction is relatively independent of the occurrence of c-mitoses is presented by the observations made during the present study that c-mitoses may occur without any c-tumours being formed. In our experiments such cases were found partly in the highest concentrations of some substances and partly in the lowest concentrations. In the former cases the absence of c-tumours may be explained by a growth-inhibiting poison effect, in the latter cases, on the other hand, the concentrations are probably below the threshold value of c-tumour effect, although they are above the threshold value of c-mitosis. In *Pisum* we did not obtain c-tumours in any concentration of the naphthalene derivatives in spite of the occurrence of c-mitosis. Similarly, EHRENBERG

and ÖSTERGREN (unpublished) did not find c-tumours in *Salix* treated with acenaphthene, although the mitoses were disturbed. These mitotic disturbances of *Salix* were not, however, full c-mitoses (EHRENBERG and ÖSTERGREN, 1942). GARRIGUES (1939), who compared the effect of chloral hydrate and colchicine on roots of *Vicia Faba*, also found that, although the two substances disturbed mitosis in a similar way, colchicine induced c-tumours but the chloral hydrate did not. We suppose that the failure of chloral hydrate to give c-tumours is dependent on the same growth-inhibiting poison effect as encountered by us in α-naphthylamine and in the strongest concentration of α-chloronaphthalene. GAVAUDAN and GAVAUDAN (1939) succeeded in certain cases in obtaining a weak c-tumour effect even with chloral hydrate, as NEMEC had already done (1904, p. 689). It should also be mentioned in this connection that growth substances have the property of inducing similar tumour-formations as the c-tumours without inducing c-mitosis (LEVAN, 1939).

As c-mitosis and c-tumours, however, as a rule accompany each other, the assumption may be expressed that these two reactions of the c-mitotic substances are caused by the influence on the same material substrate within the cell. In the next paragraph we shall more closely consider the nature of this substrate.

C. THE CONNECTION BETWEEN C-MITOTIC ACTIVITY AND WATER SOLUBILITY.

a. *The lipoid—water partition hypothesis, a comparison between the c-mitotic substances and the narcotics.* — Our experimental results concerning the threshold value of c-mitosis and the water solubility within the α-naphthalene series, which are tabulated in Table 1, are presented in a somewhat different form in Fig. 2. On the abscissa of this graph the molar concentration of the water solubility is given, and on the ordinate the corresponding values of the activity threshold. In both cases a logarithmic scale is used. Neither in the case of the solubility nor in that of the activity have the exact threshold values been determined. Our method has only permitted an enclosing of these values between certain limits that are represented in the graph. For each substance, consequently, a rectangle is drawn, within the boundary of which the exact point of the curve is situated; its absolute position is unknown. Acenaphthene, which probably does not belong to the same series, is not included in the graph.

It is immediately seen from the graph that the present experimental

data may be said to corroborate clearly the impression which we received from the paper of SHMUCK and GUSSEVA (1939), viz. that the c-mitotic activity of the substances increases at the same time as their water solubility decreases. SHMUCK and GUSSEVA's paper deals mainly

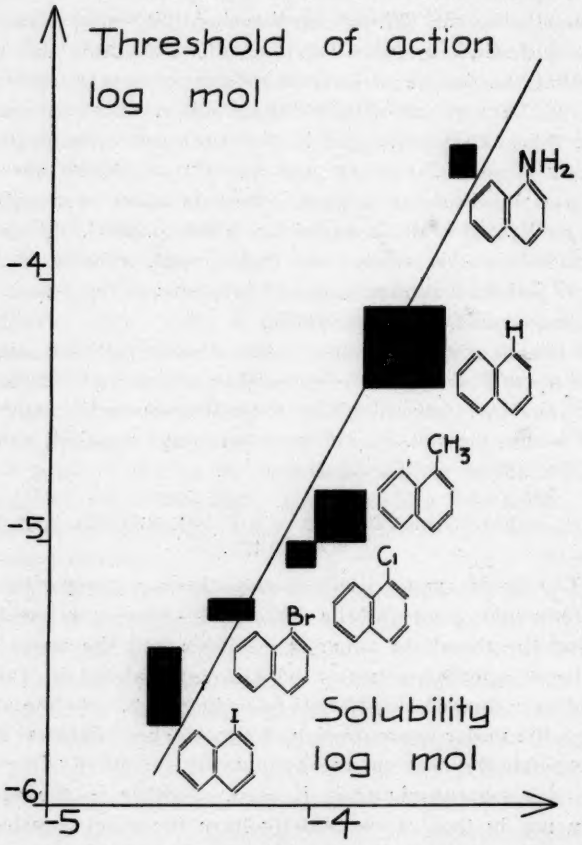

Fig. 2. The correlation between activity threshold and water solubility in the α-naphthalene series.

with naphthalene (and acenaphthene) derivatives. If these substances are tabulated according to their c-mitotic activity or inactivity, and in addition divided into substances with and substances without hydrophilic groups, the following table results:

Naphthalene and acenaphthene derivatives	Active	Inactive
with hydrophilic groups:	0	13
without » » :	13	11

According to the exact method of a 2×2 table (FISHER, 1939, pp. 100—102, paragraph 21. 02) there is only a probability of 1 : 1430 that this distribution should be due to random occurrences. It should be mentioned, however, that the evidential value of this material is not so high as it may seem at first sight. The »individuals» of the table, it should be observed, do not vary independently of each other. Thus, strong correlations in most properties must necessarily be present within the different groups of substances. The concordant behaviour of all the sulphonic acids included, for instance, might not be referable only to their similarity in solubility, it might also be due to their molecules having the same characteristic group. As the result of this examination, however, goes in the same direction as our experiments, it must be considered as a support for our view. As characteristic instances of the results of SHMUCK and GUSSEVA may be mentioned α-naphthol and α-naphthoic acid, which are inactive, while their ethers and esters are active.

Other instances in the same direction may be gathered from the literature. Thus, FAVORSKY (1939) found aniline to be inactive, while tribromoaniline was active. It is a well-known fact that the latter substance has a much lower solubility than aniline; indeed, it is obtained as a precipitate if bromine is added to an aniline solution. Another aniline derivative that is undoubtedly of lower solubility than aniline has turned out to be active, viz. phenyl urethane (LEFÈVRE, 1939). GAVAUDAN, GAVAUDAN, and DURAND (1939 b) found some c-mitotic activity in benzophenone, while MICHLER's ketone (tetramethyl diamino benzophenone), which is no doubt more easily soluble in water, was inactive. The cited authors, however, have not directed their attention to this connection between solubility and activity. That the correlation observed by us in the naphthalene derivatives (Fig. 2) cannot be a mere coincidence, is also demonstrated by preliminary *data that we have obtained from benzene, thiophene-, and cyclohexane-derivatives, and camphor and borneol, showing that in these series exactly the same type of correlation is valid.*

It is evident that this situation is very difficult to understand if it is assumed that the c-mitotic substances excercise their action in the

water phase of the cell. To explain the data observed we were therefore led to the hypothesis that *the decisive concentrations are not the concentrations of the substances in the water phase but in the lipoids of the cell.* Practically identical considerations have been suggested for the mechanism of action of narcotics. This is known as the MEYER—OVERTON lipoid theory (cf. the reviews by HENDERSON, 1930; MEYER and HEMMI, 1935; SCHAUMANN, 1938).

The concentration of the substances in the lipoids is determined by their concentration in the water phase according to the law of partition of a solute between two solvent phases:

$$\frac{C_l}{C_w} = \frac{S_l}{S_w}; \qquad C_l = C_w \frac{S_l}{S_w},$$

where C_l and C_w = the concentration in the lipoids and in the water respectively, S_l and S_w = the corresponding solubilities. The equation in the above shape is rather approximative, but it will probably suffice for our present purpose.

From this equation it follows that the concentration in the lipoid phase should be inversely correlated to the water solubility. The lipoid concentration of different substances is, however, dependent not only on variations in their water solubility, also their lipoid solubility being submitted to variations from one substance to another. This fact, of course, should considerably weaken the strength of the theoretically expected correlation between water solubility and activity of substances, but the correlation would naturally still exist. It is evident that a determination of the *partition coefficients* of the substances would give a much better measure of their activity than that given by the water solubility. In the case of narcotics a very good agreement has been obtained between the activity and the coefficients of the partition of the substances between water and suitable lipoid models as olive oil and especially oleic alcohol (MEYER and HEMMI, 1935). It was found that even very different chemical substances which had a highly variable value of the threshold concentration for their water solutions had surprisingly similar concentrations in the lipoids at these thresholds. Or, in other words, the threshold of action in the lipoids is practically the same even in very different substances. As the lipoids on which the c-mitotic substances are acting are quite unknown, it may not be easy to find a suitable model substance for a determination of partition coefficients.

Of great interest in this connection is the observation made by

RICHET (1893, cited from HENDERSON, 1930) that in many cases an obvious negative correlation is present between water solubility and narcotic activity. HENDERSON gives a table concerning this condition, from which it is seen that this rule is valid within each group of closely related substances but not between different groups. Preliminary results obtained by us in different groups of c-mitotic substances show exactly the same condition. Thus, benzene derivatives, thiophene derivatives, and camphor and borneol all show a positive correlation between water solubility and threshold, just as in the naphthalene series, with the reservation, though, that the regression lines of these series do not agree with that of the naphthalene group or with each other. (The observation that acenaphthene does not lie on the same regression line as the other naphthalene derivatives studied by us, may be due to similar conditions which make the benzene derivatives lie on an other regression line than the naphthalene substances.) Thus, in this respect there is a perfect analogy between the conditions within the narcotics and our results with the c-mitotic substances. The explanation of the inverse correlation between activity and solubility in the narcotics may be the same as given by us above.

The correlation ought to be strengthened if the solubility in lipoids is inversely correlated to the solubility in water. It is known that such an inverse correlation is often present. The introduction of hydrophilic groups as, for instance, sulphonic acid-, carboxyl-, hydroxyl- or amino-groups usually reduces the solubility of an organic substance in fatty solvents, simultaneously as the solubility in water increases. It is somewhat uncertain, though, whether one may dare to assume that this is also true in the case of a comparison between hydrocarbons and halogene-substituted hydrocarbons like those studied here. The solubility of organic substances often varies rather erratically; so great caution must be used in making any predictions in this kind of work (cf., for instance, HÜCKEL, 1941).

From Fig. 2 it is seen that when the solubility increases the threshold concentration increases still more rapidly. When the solubility exceeds a certain limit (between 300 and 1000×10^{-6} mol) the threshold value will be situated at higher concentrations than the solubility, i. e. it is impossible to obtain solutions strong enough to get any c-mitotic effect. This gives a sound explanation of the observed condition that those naphthalene derivatives which contain hydrophilic groups (e. g. carboxyl or sulphonic acid groups) were inactive (SHMUCK and GUSSEVA, 1939). One of the synthetic phytohormones is an α-substituted naphthalene

derivative (α-naphthalene acetic acid). It might perhaps have been expected that this substance in some concentration should be able to induce c-mitosis just as so many other α-naphthalene derivatives. BEAL (1938) and LEVAN (1939) did not find any activity of this kind. The regularity demonstrated above makes this quite understandable. The substance in question has a by far too high water solubility.

The same is true, although to a less degree, of α-naphthylamine. SHMUCK and GUSSEVA (1939) declared this substance to be inactive. We found, however, in accordance with FAVORSKY (1939) that it possesses a certain activity. But this activity is found only within a very narrow concentration range, and its discovery is made difficult on account of the strong poison effect, which will soon kill the roots in the active concentrations. FAVORSKY accordingly points out that the activity occurred »on the verge of cells life».

When the solubility and volatility of the substances decrease too much, a loss of activity will probably be met with. If the solubility of a substance is little enough, the result will be that water and water solutions become impenetrable barriers, checking any transport of the substance. It may be assumed that the cells have some power to destroy the c-mitotic substances. If the supply of the substance occurs too slowly, an effective concentration will never be attained in the cell. A low volatility also reduces the usefulness of the substance in treatments with vapour. SHMUCK and GUSSEVA (1939, 1940 b) found that, while 5-bromo- and 5-chloroacenaphthene were active, the 5-iodoacenaphthene gave no effect. If it is supposed that the solubility of these acenaphthene derivatives changes in an analogous manner to the halogen naphthalenes, iodoacenaphthene ought to be the one having the lowest solubility. The above reasoning may explain its inactivity.

This assumption that the c-mitotic activity would decrease if the solubility dropped below a certain limit has its clear parallel among the narcotics. MEYER and HEMMI (1935, p. 59) report that within the homologous alcohol series the threshold of activity falls in a regular manner all the way down to undecyl alcohol, but dodecyl alcohol is more inactive than expected, and tetradecyl alcohol is devoid of narcotic activity. Their explanation is the same as we suggested concerning 5-iodoacenaphthene.

The lipoid hypothesis made to explain our observational data also enables a deeper analysis of the factors controlling the size of the active concentration range. The relative range of active concentrations S_w/T_w of course equals S_l/T_l. While the threshold of action in water T_w, as is

well known, shows a very great variation from substance to substance, it may by analogy with the narcotics be assumed that the threshold in the lipoids T_l will be much less variable, perhaps even practically constant. This means that the size of the range of active concentrations is determined practically exclusively by the variation in lipoid solubility.

The difference in activity between the α- and β-derivatives of naphthalene may now be open to a deeper understanding. The explanation given above (Experimental part and II: 1) was that this difference was caused by the β-derivatives having a much smaller range of active concentrations. This difference is given a rational explanation in the light of the fact that the β-derivatives have a higher melting point than the corresponding α-derivatives. A higher melting point, as a rule, is accompanied by a lower solubility in water as well as in lipoids, and the significance of the lipoid solubility for the range of effective concentrations has just been pointed out. A lower water solubility was observed in our experiments.

The great range of active concentrations of colchicine may be due to a high solubility of this substance, not only in water but also in lipoids. Substances with a lipoid solubility equal to T_l, of course, will act only in saturated water solutions, substances with an even lower lipoid solubility will be quite inactive in all concentrations. From these considerations, again, the importance of making determinations of the partition coefficients is obvious.

There is also a possibility that there may be a variation in position of the threshold in lipoids T_l between different substances. This would mean that the activity of the substances is not controlled exclusively by their physical properties (solubilities, partition coefficients) but that their chemical nature is also important, an assumption which does not seem too improbable. Such a variation would obviously also contribute to weakening the correlation between water solubility and threshold of action. It may also account for differences in the size of the range of active concentrations.

While most of the c-mitotic substances are characterized by a very low water solubility, there is known the very prominent exception of colchicine itself. Its solubility is according to MULLER (cf. SEIDELL) 9,6 g per 100 g water at 18—22°. As may already have been understood, colchicine does not at all fall on the same regression line as the naphthalene derivatives (Fig. 2). The point corresponding to colchicine is lying far to the right of this line. The reason for this difference between colchicine and the naphthalene derivatives may be, as already

suggested, that colchicine may have a high lipoid solubility, but it may also have another threshold position in lipoids. That colchicine is soluble in lipoids is known. Thus BRUES and COHEN (1936) and HAVAS and GAL (1938) used a solution of colchicine in sesame oil in their treatments of animals.

Finally there is the possibility that colchicine interferes with the formation of the spindle at quite another point than in the lipoids. The origin of the spindle is probably a very complicated process which may be disturbed at many other instances than during reactions involving the lipoids. The agreement in the morphological picture of the colchicine effect, both as regards c-mitosis and c-tumour formation, makes it not improbable, however, that colchicine has the same mechanism of action as the other c-mitotic substances.

Of great interest in the discussion of the mechanism of action of colchicine is the fact that VERNE and VERNE—SOUBIRAN (1939), by means of a microchemical reaction, the FEULGEN—VERNE reaction, were able to establish that the colchicine even in extremely low concentrations interferes with the lipoid metabolism of the cell. It brings about an oxidation of the lipoids and acts in this respect antagonistically to carotene (provitamin A).

In view of the exceptional behaviour of colchicine in having so high a water solubility it is hardly astonishing that other exceptions, too, are found in the group of compounds chemically related to colchicine. Thus, a case is known where our solubility—activity correlation does not hold, N-acetyl-colchinol being more soluble but also more active than its methyl ether (BRUES and COHEN, 1936).

The classical lipoid theory of MEYER and OVERTON is not the only theory suggested to explain the action of narcotics. Obviously the discussions on this subject have to be also taken into account when considering the c-mitotic effect. Reviews on this topic are given by HENDERSON (1930), MEYER and HEMMI (1935), and SCHAUMANN (1938).

TRAUBE demonstrated a connection between narcotic effect and surface activity. Narcotics reduce the surface tension. Solutions with the same narcotic activity were often found to bring about the same reduction in surface tension. WARBURG (e. g. 1921) developed further the opinion of TRAUBE concerning the significance of the surface activity. Considering the tendency of narcotic substances to accumulate at interfaces, caused by their surface activity, WARBURG pointed out that they should be adsorbed on catalytically (enzymatically) working surfaces of the cell, thus blocking their function. He succeeded in de-

monstrating an activity of this kind in model experiments, in which narcotics really inhibited the catalytic combustion of various substances on the surface of charcoal.

Several of the most important arguments in favour of the adsorption theory have proved with time to be untenable (cf. MEYER and HEMMI, 1935). Many important narcotics (carbon tetrachloride, chloroform, hydrocarbons, etc.) are further almost entirely devoid of surface activity, and cannot be expected to undergo adsorption. This is probably the case also with our c-mitotic hydrocarbons.

It should be noticed, however, that BROCK, DRUCKREY, and HAMPERL (1938) found that the carcinogenic hydrocarbon benzpyrene was strongly adsorbed on various adsorbents. So an adsorption theory, after all, may not be quite excluded in our case, either.

Since the action of the narcotics does not seem to involve an adsorption, MEYER and HEMMI assume that it may instead be a question of a partition equilibrium, the active substance being partitioned between two phases. A very good agreement is obtained with partition equilibria in which lipoid substances take part, the best lipoid model being oleic alcohol. Local anaesthetics also give a rather good agreement between threshold value and partition coefficient (SCHAUMANN, 1938). While the adsorption always decreases with increasing temperature, the partition coefficient may both increase and decrease (HENDERSON, 1930). The threshold of narcotic action changes in a manner which agrees with a partition equilibrium (MEYER and HEMMI, l. c.). There has, however, been much discussion as to which theory is the more probable one; SCHAUMANN (1938) still considered the question as unsettled.

Similarities between narcotics and c-mitotic substances have already been touched upon several times. This similarity is probably more than a casual occurrence. The c-mitosis as well as the narcosis involves a reversible disconnecting of certain vital functions, while other functions go on normally. Those functions are disconnected which lead to the formation of the spindle. NEBEL and RUTTLE (1938 a) have shown that the plasmatic currents go on normally in cells during c-mitosis. LUDFORD (1936) found in tissue cultures under normal conditions that dividing cells were vital-stained by methylene blue much fainter than resting cells, depending on a more intense reduction of the dye. Cells undergoing mitosis under the influence of colchicine behaved in the same manner in this respect as under normal conditions. The growth of the cells also continues under the influence of c-mitotic

substances (as evidenced by the c-tumour reaction). *The c-mitotic effect might be regarded as a kind of narcosis.*

In favour of such an assumption speaks the fact that several workers have succeeded in inducing c-mitosis-like disturbances with narcotics. Among such cases may be mentioned chloral hydrate (e. g. NĚMEC, 1904; VAN REGEMORTER, 1926; GARRIGUES, 1939), ether (POLITZER, 1931; ROSENFELD, 1932), and phenyl urethane (LEFÈVRE, 1939). According to preliminary results obtained by us, benzene, which is also known to have narcotic properties (HENDERSON, 1930, p. 190), also has clear c-mitotic effects. Thymol, reported to have narcotic activity by MEYER and HEMMI (1935, p. 58), was found by VILLARS (1940) to produce c-mitosis.

A detailed examination of the effect of narcotics would undoubtedly increase the number of such instances. Alcohol deserves a special investigation, as we found it able to counteract the effect of colchicine in *Allium*, while POLITZER obtained c-mitosis-like disturbances in *Salamandra* after treatment with alcohol.

The connection between c-mitotic substances and narcotics is also of interest in the attempts to understand the origin of c-tumours. BORGSTRÖM (1939 a), studying the characteristic response of plants to ethylene (tumefactions, etc.), formed the opinion that this substance and other substances having a similar effect, acetylene, propylene, carbon monoxide, and ether, were operative through their narcotic effect. This effect was considered to cause an increase in permeability of the phloem followed by a streaming out in all directions of the growth hormones. In addition the ethylene brought about an increased sensitivity of the plants to the hormones. ZIMMERMAN (1935) could narcotize insects, centipedes, and plants with ethylene, acetylene, propylene, and carbon monoxide. In insects carbon monoxide was a less poisonous narcotic than ether and chloroform. Changes in the permeability have often been studied and discussed in connection with the action of narcotics (HENDERSON, 1930; SCHAUMANN, 1938). The similarity demonstrated by us in the mechanism of action between c-mitotic substances and narcotics makes it very probable that *the c-tumour reaction is identical with the ethylene effect.* It should be noticed that this assumption may be correct even independently of the opinion of BORGSTRÖM concerning the mechanism of the ethylene effect. Ethylene and ether may induce epinasty (BORGSTRÖM, 1939 a). The same effect has been attained by colchicine treatment (HAVAS, 1938). HAVAS, however, assumed that the colchicine had no auxin

effect of its own, as the classical pea test method of WENT gave entirely negative results. It would be more probable that its action took place over the auxins.

Ethylene, however, does not induce c-mitosis (KOSTOFF, 1940 a; LEVAN, unpublished). As demonstrated above, the c-tumour and c-mitosis do not always accompany each other. They may have different threshold values. It is possible that higher concentrations of ethylene than have been tested might give c-mitotic disturbances.

It is a well-known fact that the permeability of the cells, which is of great interest in connection with the present problems, is regulated to a certain degree by the lipoids which are gathered in the plasmatic membrane of the cells. This view was first presented by OVERTON. The latest opinions on cell permeability (FREY-WYSSLING, 1938 a, p. 153) also acknowledge the importance of lipoids, even if these substances are not considered to be solely decisive. The significance of the lipoids is also seen from the work of BALLENTINE and PARPART (1940), who could change the permeability of red blood corpuscles by means of a lipolytic enzyme, splitting off fatty acids in the »phospholipids» of the plasm membrane. A proteolytic enzyme had no such influence. WOLPERS (1941) was able to demonstrate with the aid of the electron microscope that the erythrocyte membrane consists of a micellar protein-»gerüst», filled up with lipoids.

Both the c-mitosis and the c-tumour-formation may be regarded as depending on a narcosis of superior, regulating functions of the cell. The c-mitosis is a narcosis of those factors which govern the growth by cell division, the c-tumour-formation is a narcosis of those factors which govern the growth by cell-tension. Concerning this latter process it should be noted that instead of the normal, limited tension in a determined usually polar direction, an unimpeded tension in all possible directions asserts itself under the c-tumour growth, tending to make the cells isodiametric.

The fact that these two types of narcosis may occur to a certain degree independently of each other may indicate that it is a question of an influence on different lipoid systems of the cell. As is well known, different narcotics show a varying tendency to influence different nervous centra. MEYER and HEMMI (1935, p. 58) suppose that this is due to different narcotics showing special affinities to different lipoids. Different lipoid-structures may be in charge of different cellular mechanisms.

Thus, it seems natural to assume that cell tension may be especially

dependent on the conditions of the lipoid-rich plasm membrane lying immediately plastered to the cell walls, and on its permeability to hormones. The formation of the nuclear spindle, on the other hand, is probably controlled by some lipoid-containing substrate in the interior of the cell (below are discussed the chondriosomes and the centromeres).

Differences in the lipoids between species may cause such differences in reaction as were found in *Allium* and *Pisum*. It will be recalled that the naphthalene derivatives did not induce any c-tumours in *Pisum* in spite of the occurrence of c-mitosis. According to MEYER and HEMMI, the minimum concentration of narcotics which must be attained in the lipoids of the cells if narcosis is to be obtained is different in different species (l. c., p. 59). In *Epilobium* differences in lipoids have been demonstrated by permeability studies. They showed plasmatic inheritance as observed after 12 generations of back crosses (DELLINGSHAUSEN, 1935).

The connection between water solubility and activity of the naphthalene derivatives in *Pisum* may probably be expressed by a regression line parallel to that of *Allium* and located to the left of this line (Fig. 2). In this connection account should also be taken of the evident differences in reaction to colchicine between higher plants, on one side, and fungi and algae, on the other.

Yeast, for instance, is not influenced even by very strong colchicine concentrations. The most pronounced reaction in yeast to c-mitotic substances is induced by camphor. It is, however, questionable whether a real c-mitosis is concerned in this case. The green alga *Ulva* is very sensitive to treatment with acenaphthene, while it is dubious whether colchicine has any specific action at all (LEVAN and LEVRING, 1942). It is also remarkable that *Colchicum* is very sensitive to acenaphthene, although it is completely immune to colchicine (LEVAN, 1940 a). The antagonism of certain substances to colchicine must also be considered in this connection. It is possible that the presence of such substances in the cells might induce immunity or reduced sensitivity to colchicine or other c-mitotic substances.

b. *An alternative interpretation?* — Thus, as we have seen, a lipoid hypothesis may be able to bring about a considerably deepened understanding of many of the peculiarities of the c-mitotic reaction. Still, there are some unsatisfactory features in the picture. There is, for instance, the question of *how the substances present in the lipoids actually produce their effect.* It might be suggested that they take part

in some chemical reaction going on in the lipoids. An argument against this assumption is that in such a case their activity would have been much more dependent on a specific chemical structure. Hydrocarbons like acenaphthene undoubtedly have no great chemical reactivity. In the case of narcotics with their even more varying chemical structure such an assumption is, of course, quite improbable. MEYER and HEMMI (1935) seem to be aware of this difficulty in the lipoid theory; they state concerning this theory that it »... kaum mehr als Theorie bezeichnet werden kann, sondern nur das Tatsachenmaterial in Form eines Gesetzes oder einer Regel zusammenfasst» (l. c., p. 59).

It is undoubtedly closer at hand to think of a purely physical action. The theory of WARBURG already mentioned, with its assumption of a blocking of surface catalysis in this respect, offers a simple explanation. The theory of WARBURG, however, suffers from considerable drawbacks in other respects (cf. HENDERSON, 1930, and MEYER and HEMMI, 1935). SCHAUMANN (1938) considers it as still unsettled which of the theories of narcosis is the most probable one.

A sort of lipoid theory may be justified by assuming changes in permeability produced by a solution in (or an adsorption on) lipoid-containing plasm membranes. Changes in permeability have often been observed or discussed in studies on the effects of narcotics. The c-mitosis, of course, may also be produced through changes in permeability, although it is certainly difficult to see how. For the understanding of such processes it is more necessary to study the influence of organic substances on lipophilic colloids than to study their partition between well defined water and lipoid phases. In this connection the researches of BUNGENBERG DE JONG and his collaborators on lipophilic coacervates are of special interest. These studies may even give a plausible explanation for the antagonism of ethyl alcohol to colchicine.

It was found by BUNGENBERG DE JONG and SAUBERT (1937 a, b and c), BUNGENBERG DE JONG, BOOY, and SAUBERT (1937, 1938 a and b), BUNGENBERG DE JONG, SAUBERT, and BOOY (1938 a and b), and KOETS and BUNGENBERG DE JONG (1938) that organic non-electrolytes could produce two different effects on oleate and phosphatid coacervates, viz. either a swelling or a shrinkage. Hydrocarbons produced a shrinkage by attracting the long hydrocarbon chains of the coacervate substance with VAN DER WAALS' forces. Substances containing hydrophilic groups, on the other hand, could produce a swelling by increasing the hydration of the coacervate. Whether the shrinking

or the swelling properties of a substance were to prevail was dependent on the relative importance of hydrophilic groups and hydrocarbon residue of the substance in question. Thus, in the alcohol series the lower members produce swelling, but with an increasing number of carbon atoms the effect is changed to a shrinkage, which grows stronger and stronger. Thus, it is found that the lower alcohols have an effect antagonistic to the hydrocarbons. This may give an explanation of the antagonism between ethyl alcohol and colchicine, colchicine acting in the same manner as the hydrocarbons naphthalene and acenaphthene in our experiments. (Experimental work along the line indicated by this hypothesis is now being continued by ÖSTERGREN.) The shrinking activity was regularly found to increase with decreasing hydrophilic properties of the substances, a rule which may account for the negative correlation between solubility and threshold of action in our experiments. Thus, aromatic hydrocarbon radicals were found to have a weaker shrinking power than aliphatic, and they are also more hydrophilic (benzene being more soluble in water than hexane, for instance). It was also found that the introduction of halogens strengthened the shrinking activity of alcohols.

The properties of the plasm membrane have also been studied by some workers from similar points of view to those presented by BUNGENBERG DE JONG. SAUBERT (1937) considered it to be an oriented structure, like a liquid crystal, consequently differing somewhat from the liquid coacervates.

An action on lipoid coacervates should undoubtedly be kept in mind as an explanation alternative to the distribution between two distinct phases. The result of such a mechanism might not be too different from that expected from a partition hypothesis. (As it may not be able, however, to account for the good agreement between partition coefficients and activity of narcotics, we do not venture to suggest it as a theory of *narcosis*.) Further, it may quite well be possible that the substrate of the c-mitotic action is not the plasm membrane but some intracellular coacervate; below we suggest as a possibility the mitochondria.

Summing up our view concerning the mechanism of action of c-mitotic substances, it can be stated that these compounds *do not produce their effect because of their chemical properties, but chiefly or exclusively because of their physical properties.* In this respect they are related to narcotics. C-mitosis also shows other similarities to narcosis. *Probably any substance the physical properties of which fulfil*

certain not very strict conditions would be a c-mitotic substance. Chemically a c-mitotic substance may even be quite indifferent. The fact that hydrocarbons like acenaphthene belong to the most efficient substances may indicate this. Colchicine, however, may be an exception where chemical properties are of more importance.

c. *The cell organ subjected to action.* — It may be speculated on as to what parts of the cell may be subjected to the action of the c-mitotic substances. Firstly it should be pointed out that the whole of the plasm is impregnated with lipoids (this is stressed, for instance, by BENSLEY, 1937); so there is, perhaps, no need to search for a special cell organ. There is, however, a type of structure that is known to be especially rich in lipoids, viz. the mitochondria.

There are few chapters of biology in which so many contradictory and insufficiently founded assertions stand against each other as concerning the mitochondria (see, for instance, the review of NEWCOMER, 1940). Especially the discussion as to whether they may originate *de novo*, and whether they have any ontogenic connection with the plastids, makes a discouraging impression.

Opinions have been presented that the mitochondria have essential functions to fill in the metabolism of the cell. Their ubiquity in all organisms cannot of course be regarded as conclusive evidence of this. Neither would this be the case with their increase in number in cells with lively metabolism, their gathering around nutrition vacuoles and other such morphological observations (cf. HORNING, 1933), were these observations not supported in other ways.

So much the greater significance must be ascribed to that evidence which is founded on direct microchemical reactions. JOYET-LAVERGNE (1938, and earlier papers), who could directly demonstrate their power to oxidize and reduce various substances, is of opinion that the mitochondria have a function in the respiratory process. He was also able to show with the aid of micro-chemical methods that they contain vitamin A (1937 and 1938). The occurrence of vitamin A in the mitochondria was confirmed by means of the fluorescence microscope by GRAFFI and MAAS (1940). This localisation of vitamin A was also made by GOERNER (1938) and GOERNER and GOERNER (1939).

MARSTON (1923) presented the theory that the mitochondria contained proteolytic enzymes and took an important part in the protein synthesis. These conclusions were drawn on the basis of their specific stainability with Janus green B and related stains, which were shown to act as specific precipitating agents for such enzymes. ROBERTSON (1926) assumed that the lipoids of the chondriosomes had as their particular function an orientating influence on the amino-acids in order to get the —COOH and —NH_2 groups approached to each other, whereby the protein synthesis would be facilitated. MARSTON (1926), in fact, succeeded in accelerating the synthetic effect of proteolytic enzymes by adding lipoid emulsions.

LANGENBECK (1933) found that certain substances containing amino-groups are good models for the natural carboxylases. His enzyme models, however, were inactive in water, and had their strongest effect in hydroxyl-containing organic solvents. He obtained one preparation that was also active in water by using an emulsion of methyl-salicylate + lecithin as water-insoluble carrier for his ferment model. He presumes: »Vielleicht ist auch die Carboxylase in einem kolloidalen

Träger gelöst, der in ähnlicher Weise die schädliche Wirkung des Wassers ausschaltet».

BENSLEY (1937) studied the chemical composition of the mitochondria. He assumed them to have coacervate structure, and he was rather sceptical as to any specificity in their nature. [On the other hand, their specific staining reactions mentioned above (MARSTON, 1923) must be considered to speak in favour of such a specificity.] It may be expected, we think, that the c-mitotic hydrocarbons are able to provoke similar condensations in the mitochondria as in the coacervates of BUNGENBERG DE JONG. These changes would be able to interfere with the course of enzymatic functions, maybe a rebuilding of the protein molecules, necessary for the formation of the nuclear spindle. GRAFFI (1940 b) refers to many other authors that have ascribed to the mitochondria important functions in various respects.

The chloroplasts, which are the seat of a very important catalytic process, the photosynthesis, also contain lipoids. According to FREY-WYSSLING (1938 a and b), these lipoids are arranged in definite layers, into which the phytol residues of the chlorophyll molecules are inserted. The lipoids also contain the carotinoids of the chloroplasts. It may be suggested that the function of the lipoids here is to bring about an orientation of the chlorophyll molecules in a definite manner, thus, as it seems to us, a similar function to that ascribed to the lipoids of the mitochondria by ROBERTSON (1926). The lipoids are perhaps especially adapted for such directing and orientating functions, among other reasons because of their great tendency to form oriented systems (e. g. myeline-structures). The plastids are also the seat of other metabolic activities as, for instance, the formation of starch grains in the leucoplasts.

Another cell organ which may be suggested as substrate for the c-mitotic action is the centromeric parts of the chromosomes. Their importance for the normal course of chromosome movements is well-known. Perhaps they have important functions in the organisation of the spindle, too. That, however, is quite hypothetical. SCHRADER (1936) and BENOIT and KEHL (1939) find a similarity in staining reactions between the centromeres and the mitochondria. Now it is known that these latter besides containing proteins are also characterized by their content of lipoids. It is, then, not very far-fetched to conjecture, as do BENOIT and KEHL, that the centromeres may also contain lipoids. A similarity in structure between mitochondria and centromeres is perhaps not so unbelievable, considering that the mitochondria also are assumed by many cytologists to be self-reproducing bodies.

D. SIMILARITY IN ACTION BETWEEN C-MITOTIC AND CARCINOGENIC SUBSTANCES.

SHMUCK (1938) suggested that colchicine is related chemically to the carcinogenic substances. He pointed out that colchicine is a phenanthrene derivative like important carcinogenic hydrocarbons. The specific properties of colchicine might according to him be caused by its being a derivative of a condensed polycyclic hydrocarbon. This assumption made him also test other substances with systems of condensed rings, which led to the discovery of the strong c-mitotic effects

of acenaphthene. SHMUCK and co-workers in later papers developed this thought further (SHMUCK and GUSSEVA, 1939, 1940 c; SHMUCK, GUSSEVA, and ILJIN, 1939). As already mentioned, SHMUCK and GUSSEVA (1939) compared a whole series of naphthalene and acenaphthene derivatives with similar derivatives of carcinogenic hydrocarbons known in literature. They found a parallel extinction of the c-mitotic and the carcinogenic activity, if certain substituents were introduced in the molecules of the c-mitotic and the carcinogenic substances respectively. They drew the conclusion (1940 c) that this similarity is more than a random occurrence, and suggested »the existence of a general mechanism behind these biological phenomena in plants and animals», without stating, however, how the mechanism was to work.

Our observation of a connection between water solubility and activity of the c-mitotic substances prompted us to inquire whether similar conditions were also valid among the carcinogenic substances. The similarity between the two groups of substances pointed out by SHMUCK and GUSSEVA might in that case be caused by the condition that both types of substances acted on or within the same substrate of the cell, viz. the lipoids. The results cited by SHMUCK and GUSSEVA (1939) clearly indicated that hydrophilic groups have a tendency to extinguish the carcinogenic activity.

The most active among the carcinogenic substances are 9 : 10-dimethyl-1 : 2-benz-anthracene (COOK, 1940), methylcholanthrene, and benzpyrene. These substances and many similar carcinogenics are hydrocarbons and practically insoluble in water, but relatively easily soluble in lipoids. The water solubility of methylcholanthrene is according to HOLLAENDER, COLE, and BRACKETT (1939) 1 part in 10^8. COOK *et alii* (1937) point out that simple carboxylic acids and sulphonic acids of 1 : 2 : 5 : 6-dibenzanthracene are devoid of carcinogenic activity. WINDAUS and RENNHAK (1937) and ROSSNER (1937) were of opinion that it would be of special interest to produce water-soluble derivatives of benzpyrene and methyl cholanthrene, but they found that all their water-soluble compounds were inactive. FIESER (1938) says: »Clearly no general rules are established by the few results on record, but it may be said that the oxygenated compounds thus far examined are either inactive, or in comparison with the most potently carcinogenic hydrocarbons of the group, but weakly active» (l. c., p. 103). Activity was found, however, in one primary amine. COOK and KENNAWAY (1938) considered the complete loss of carcinogenic power resulting from the introduction of hydroxyl groups into the molecules of two very active

carcinogenic hydrocarbons to be of considerable interest. HADDOW and ROBINSON (1939) had demonstrated a connection between growth-inhibiting power and carcinogenic activity in polycyclic hydrocarbons and other substances, and BADGER and COOK (1940) found that the introduction of hydroxyl and carbonyl groups into carcinogenic hydrocarbons was accompanied by a marked loss of growth-inhibiting activity.

The observation that the water-soluble salts formed by the endo-α-β-succinic acids originating as additive compounds from maleic anhydride and carcinogenic hydrocarbons possess carcinogenic activity (COOK *et alii*, 1937; FIESER *et alii*, 1937), would seem to disturb the picture. These compounds, however, as a matter of fact give additional support to our view, as it was found that the active endo-succinic acids are able again to dissociate into maleic acid and hydrocarbon. The endo-succinic acid of the very powerful carcinogenic substance 9 : 10-dimethyl-1 : 2-dibenzanthracene, which does not dissociate, is inactive (PARSONS and WARREN, cited from BOYLAND, 1940).

Carcinogenic activity is also known from quite another group of substances, viz. certain azo-dyes. They are characterized by an organ-specificity in their carcinogenic action, tumours being provoked especially in the liver and in the urinary bladder. These substances are somewhat more hydrophilic than the hydrocarbons discussed above, as in most cases they are furnished with amino-groups. The same regularity seems to be present, however, also in this group of carcinogenic substances. According to BROCK, DRUCKREY, and HAMPERL (1940), their activity disappears at the substitution with oxy-groups.

One highly water-soluble substance (2 %) is known, however, which nevertheless is a potent carcinogenic agent, viz. the quinoline derivative called Styryl 430 by BROWNING, GULBRANSEN, and NIVEN (1936). As this substance belongs to quite another chemical group, no direct comparisons can be made with the earlier mentioned carcinogenic substances. Styryl 430 may be regarded as having an analogous position among the carcinogenic substances as colchicine has among the c-mitotic substances.

Explanations of this inactivation of the carcinogenic substances by the introduction of certain radicals have been attempted from time to time. Thus, BUTENANDT (1938) points out that the substances become more reactive by the introduction of functional groups. This condition should make it easier for the organism to get rid of the substances. A

similar reasoning is advanced by BROCK, DRUCKREY, and HAMPERL (1940) concerning the azo-dyes.

The conditions discussed above show an evident similarity to the situation in c-mitotic substances and in narcotics. We think it possible, therefore, that the carcinogenic substances exercise their action in or through the lipoids of the cell. Thus, the carcinogenic effect may be a third type of specific growth response, induced through a lipoid system. Just as the c-tumour reaction may be regarded as a disturbance of the normal volume growth of the cell, and the c-mitotic reaction as a disturbance of the growth through mitoses, so may the carcinogenic reaction be regarded as an interference with the correlative cell growth.

The question may now be raised as to what part of the cell the carcinogenic action may be localized and in what manner it is exercised. Regarding the carcinogenic hydrocarbons there is a possibility of directly observing where in the cell they get stored. Thanks to the strong fluorescence of these substances in ultra-violet light, GRAFFI (1939, 1940 a, 1941 a and b) and GRAFFI and MAAS (1940), with the aid of the fluorescence microscope, had no difficulty in determining their localisation within the cells. It was found that the substances in question were chiefly stored in the lipoids, e. g. in fat globules, in lipochondria, and in mitochondria. They also showed a weak and more diffuse distribution in the cytoplasm. Within the nucleus, on the other hand, they were detected exclusively in the nucleolus, though they were not always found even here. The authors were able to demonstrate with certainty that the mitochondria took up the substances, because the same bodies were found to be vital-stained with the specific mitochondrial dye Janus green. On the basis of these results the hypothesis was presented that the carcinogenic substances are acting on the mitochondria. The importance of the mitochondria for different metabolic processes was stressed, as also their possible property of being carriers of the plasmatic inheritance (GRAFFI, 1940 a and b, and DITTMAR, 1941).

Several investigators mention the possibility that the mitochondria might be the carriers of plasmatic genes, but many also reject this view (cf. NEWCOMER, 1940). The lipoids in any case cannot very well be regarded as gene-carriers. BENSLEY (1937), for instance, stressed that the lipoids of mitochondria were by no means specific. Mitochondria, however, also contain proteins, and concerning them such assumptions must be considered as more plausible. The lipoids are perhaps only tools, which the more specific components of mitochondria must employ for the execution of certain functions.

This problem is closely connected with the question whether the mitochondria are always self-reproducing bodies, or whether they sometimes originate from the plasm *de novo*. We think that in this discussion it should be considered that any observations that mitochondria may disappear and then be formed again do not really exclude the possibility that they may be, or may contain, self-reproducing bodies with a permanent genetical continuity. It is quite possible that they may pass through stages or conditions during which they are invisible. This is true with the

chromosomes, which are visible only during the different stages of mitosis and meiosis. Yet we know that the chromosomes maintain a perfect morpho-genetic continuity through the resting stages. Some authors think that the plastids in plants originate from the mitochondria. In that case the view of their genetic continuity would be ensured, since it has been satisfactorily confirmed that the plastids carry genetic factors. YUASA (1939), however, who was able to follow plastids and mitochondria through the entire life cycle in a fern, was of opinion that mitochondria and plastids were independent of each other, both being reproduced by division. He could distinguish the mitochondria from the plastids at all stages by means of specific staining of the mitochondria with Janus green.

Another thing to be considered in a discussion of permanent plasmatic determinants is the fact that two important groups of organisms, viz. Bacteria and Cyanophyceae have their genotype organized exclusively on a plasmatic basis. DARLINGTON (1939) puts forward the presumption that the plasmone of higher organisms might be directly derived from the genotype present before the evolution of a nucleus.

Even if the mitochondria are not absolutely independent of influence from the nucleus, they may nevertheless own a certain autonomy. There is a whole group of phenomena, the so-called dauermodifications, which are inherited for some time and then disappear. They exhibit a plasmatic inheritance, i. e. they are carried over mainly on the female side. It is close at hand to assume some kind of self-reproducing bodies as substrate for them.

Dauermodifications may originate spontaneously. Thus, in one very well-known case SIRKS (1938) obtained a plasmatic »bud-variation» in *Phaseolus*. A couple of pods deviated by their smaller size. The difference was inherited exclusively on the female side. The progeny turned more and more normal for each new generation. In the eighth generation the most deviating of the two types had reverted to the normal type.

Dauermodifications may also be induced experimentally by different external agents. It is of very great interest in this connection that several cases are known in which c-mitotic and carcinogenic substances have given rise to certain or suspected dauermodifications. Thus, HOFMANN (1927) succeeded in inducing dauermodifications in *Phaseolus* by chloral hydrate, which is a c-mitotic substance. The aberrant types were maintained through six generations. During the next few years, now that many workers are studying the production of polyploid plants by colchicine, it is probable that many new instances of dauermodifications induced by colchicine will become known. Anyone who has worked for some time with colchicine treatments on different plant materials has undoubtedly observed that, besides the polyploids which appear, also aberrant types with unchanged chromosome number emerge. These new types are often robust and dark-green forms. Thus JENSEN and LEVAN (unpublished) obtained after colchicine treatments on different materials an evident growth stimulation, which in some cases indicated a more profound cause. After treatment of tetraploid sugar beet seed with colchicine a stimulation was obtained that was maintained all through the vegetation period and was still quite conspicuous at the harvest. The average weight (root and top) of the treated beets was 1,7 kg, while the untreated beets weighed 1,0 kg. The fact was checked that the chromosome numbers of the treated beets were unchanged. Colchicine treatments of different buds of one individual of *Ligustrum vulgare*

atrovirens gave rise to two deviating types, which were cloned and propagated as cuttings for several generations. The deviating types were coarser and had broader and thicker leaves than the original shrub, untreated branches of which were cloned and cultured as controls. Also here no changes in chromosome number had occurred. JENSEN has obtained similar results from several other materials, e. g. in cereals, in *Ribes*, and in fruit trees.

The deviating yeast forms produced by DODGE and DODGE (1937) and BAUCH (1941 a, b, 1942 a, b) after treatment with c-mitotic and carcinogenic substances as well as phytohormones were often giant forms, possibly in some cases representing dauermodifications. If in BAUCH's experiments all deviating forms had been tetraploids (which is by no means claimed by BAUCH), they would have been identical, as they were derived from the same original clone. Several differences are reported, however. Although aneuploidy might have played a part, it seems more feasible to think of dauermodifications. When considering the origin of these aberrant yeast races it is of special interest to notice that GRAFFI (1941 b) made the observation that yeast cells treated with benzpyrene have this substance localized to the cytoplasmic lipoid-containing volutin granules. These granules according to CASPERSSON (1941) also contain nucleic acids of the ribose type (i. e. the type characteristic of the cytoplasm). CASPERSSON suggests that these granules have important functions in the synthesis of the cytoplasm proteins. GRAFFI suggests that they may be homologous with mitochondria of other organisms.

Among animals, colchicine has produced aberrant types in *Daphnia* (DUNHAM and REID, 1939; DUNHAM and BANTA, 1940). They originated in a *Daphnia* clone, and their chromosome conditions were not clearly traceable, but the occurrence of tetraploidy was very improbable. In some cases reversions to the original type were observed. The explanation may be the occurrence of supernumerary chromosomes which were later eliminated.

An especially interesting case of dauermodifications induced by the carcinogenic substance benzpyrene is reported by MOTTRAM (1940). He treated *Paramaecium* with 1 : 50000 and 1 : 100000 solutions of benzpyrene. Besides a general growth stimulation also the origin of aberrant types was brought about. In the stronger solution deviating types appeared after a treatment of 62 days, in the weaker solution after 122 days. The phenomenon was repeated on the 83rd and 165th day respectively. No aberrant types were found in the controls. The aberrants exhibited a wide morphological variation, some were giants, many times the normal size, some were midgets, some »siamese twins», some were no more than knobbly monsters. Some types kept constant generation after generation through several months, others returned to normal appearance. MOTTRAM stresses the resemblance to the origin of tumours. In a paper which we regret has not been available to us, MOTTRAM (1942), on the strength of results of this type, seems to have proposed a cytoplasmic hypothesis of cancer.

The carcinogenic substances have induced what may be dauermodifications among multicellular animals as well. DRUCKREY (1942) reports one case in which treatment with benzpyrene of eggs of *Strongylocentrotus* led to the origin of a giant-formed larva. It is especially stated that no mitotic disturbances were observed. Various authors have met with the formation of deviating cell-types, often giant cells, after treatment of tissue cultures with carcinogenic substances (e. g. MAUER, 1938).

As it has been established that dauermodifications may be induced both by c-mitotic and carcinogenic substances, and as these substances according to our opinion act in the lipoids of the cell, it may not be regarded as far-fetched to try to interpret the cancer phenomenon as depending on some change of the dauermodification type in some self-reproducing lipoid-containing structure of the plasm, such as perhaps, which is most likely, in the mitochondria. The often discussed theory of BAUER (1928) that cancer is due to gene mutations or some other change within the genome does not need to be entirely excluded by the view presented here; cancer may not in all cases have the same origin. GRAFFI (1940 b) and DITTMAR (1941) also mention the word dauermodification in their discussion of the origin of cancer.

Cancer may be described as a disturbance of the normal ontogenetic development, the correlative growth. It is known that the various kinds of cells which are developed during tissue differentiation under normal conditions very keenly keep many of their characteristic qualities when transplanted into tissue culture. The same is the case with cancer cells, which has been pointed out as supporting the mutation theory (e. g. DECKNER, 1938). A rather fantastic hypothesis has been put forward by THOMPSON (1938) that normal tissue differentiation is guided by gene mutations that follow each other in a regular sequence, different in different tissues. It seems more probable that the differentiation is accompanied by plasmatic changes; maybe an analogy should be sought among dauermodifications rather than among mutations. The spontaneous cancer might have its correspondence in such spontaneous dauermodifications as SIRKS's *Phaseolus* case.

Is it possible to suggest anything about the mechanism of action of the carcinogenic substances? Various opinions have been presented. DRUCKREY (1938) considered it difficult to explain the strong effect of these substances on a chemical basis. Their chemical nature made it unlikely, according to him, that they would take part in any chemical reactions. »Eher wären die *physikalischen* Eigenschaften geeignet den Wirkungsmechanismus zu erklären, z. B. die Löslichkeit in Körperflüssigkeiten und Lipoiden oder die Fähigkeit an Strukturen zu haften, Eigenschaften also, die in Wirkungsmechanismus der Narkotica eine Rolle spielen» (l. c., p. 184). The solubility and adsorption properties of benzpyrene were studied by BROCK, DRUCKREY, and HAMPERL (1938). The suggestions made by us above concerning the similarities between c-mitotic and carcinogenic substances also stress the importance of such properties, the c-mitotic substances showing close relations to narcotics.

It seems probable, however, that such properties do not alone suffice to explain the activity. Mutation-like chemical changes in a self-reproducing substrate of the cell (such as the mitochondria?) would *a priori* be expected to require an activation by energy just like all chemical changes. Genic mutations are induced by X-rays in such a manner. Starting from valency-chemical considerations, SCHMIDT (1941) presented the hypothesis that the structure of the carcinogenic hydrocarbons bestowed on them an especially low excitement energy (»Anregungsenergie»). They should therefore be especially suited for transferring to other molecules energy quanta which would be required for the induction of mutations. These properties could be correlated to certain spectrographic qualities of the substances. According to SCHMIDT the carcinogenic substances, because of this, should also be expected

to have a high chemical reactivity. The presence of a connection between chemical reactivity and carcinogenic qualities has also been stressed by FIESER (1938).

WEIGERT and MOTTRAM (1940) made similar suggestions. They established that benzpyrene occurred under different enantiomorphous modifications, stable within different temperature ranges. They found that cholesterol seemed temporarily to stabilize the »blue» form of benzpyrene, otherwise unstable at normal temperature. This form was expected to change spontaneously into the »green» form with release of energy. This may account for its biological activity. Such a temporary stabilisation of the unstable blue form of benzpyrene might be brought about within the living cells by the lipoids, as these authors suggested.

In fowls virus tumours (evidence: filterable transplantation) are quite common (cf. BUTENANDT, 1940), in mammals, on the other hand, they are very rare. A papilloma of virus origin is known, however, in the rabbit (DANEEL, 1941). There are even some indications that chemically induced tumours may be propagated cell-free, i. e. that there might be a *de novo* origin of virus under chemical influence (MCINTOSH and SELBIE, 1939, on fowls; cited from BUTENANDT, 1940). There is a possibility, however, that the virus was already present at the start of the experiment but was made active by the treatment. GRAFFI (1940 b) also refers to a number of other similar cases. Of interest in this connection are also the results of DANEEL (1941), who was able to extract a papilloma virus from the skin of perfectly healthy rabbits after long storage of the skin-pieces in aqueous glycerine solutions in cold.

GRAFFI (1940 a and b) suggested that the filterable transplantation of tumours might be due to a transference of malignant mitochondria. The virus of the rabbit papilloma studied by DANEEL (1941) was a high molecular protein. We think, however, that even a lipoid-free transference of tumours may not disprove the mitochondria hypothesis. The specific component of the mitochondria would probably be its proteins. According to HORNING (1933), mitochondria are able to pass from one cell into another. GRAFFI (1940 b) has collected a great many arguments meant to support the view that filterable cancer is transferred by irreversibly changed mitochondria. Mitochondria are perhaps aggregates of plasmatic »genes», and a virus may be such a gene that has been changed through mutation. How such irreversible changes are related to the reversible changes known as dauermodifications, is, however, a question to which we can give no answer.

From the above discussion it is seen that there are some grounds for assuming that, at any rate in certain cases, cancer may be caused by »mutations» within »plasmatic genes», either spontaneous or induced by chemical or other influences. Cancer may be started by radium, X-rays and ultra violet light, which has been regarded as supporting the mutation theory. This fact may be taken as supporting to an equal extent the possibility discussed here. Changes in plasm-genes are likely to be induced as well by the lipoid-soluble carcinogenic substances as by different kinds of radiations.

Temperature, as is well known, is also able to induce gene mutations; the kinetic energy of heat can supply the activating energy for the chemical change. Spontaneous mutations, too, seem to be produced through the heat oscillations of molecules (TIMOFEEFF-RESSOVSKY and ZIMMER, 1941). Heat, however, may induce dauermodifications as well (JOLLOS, 1935, in *Drosophila*). Such similarities between mutations and dauermodifications may indicate that they are related

phenomena, dauermodifications being mutation-like changes of plasm-genes. The suggestion that cancer should arise through changes in cytoplasmatic granules seems to have been suggested first by SKWORZOFF, 1902, and SCHLATER, 1909; cited from GRAFFI, 1940 b, p. 517.

The most obvious effect of the c-mitotic substances is the cytological induction of c-mitosis. A brief discussion follows as to whether the carcinogenic substances have any specific cytological effect. It may at once be stated that the carcinogenic substances are no effective c-mitotic agents. In numerous and varying materials it has been found that nuclear divisions go on normally, or even at a stimulated rate, under the influence of carcinogenic substances. KOSTOFF (1940 a) treated seedlings of cereals with methylcholanthrene and benzpyrene without noticing any disturbances. Many investigations, on the other hand, show that the mitoses in tissues treated with carcinogenic substances or in spontaneous tumours may often exhibit rather conspicuous disturbances. These disturbances, however, are usually rather of the nature of secondary effects, caused by the unregulated cell-growth in the cancer tissue, and not, as in the c-mitosis, a primary cell response to the substance in question.

Thus, asymmetric and multipolar spindles are common. WINGE (1930) has illustrated such disturbances in tar carcinomata of mice. MAUER (1938) has observed similar things in tissue cultures treated with carcinogenic substances. Similar observations have also been made in spontaneous cancer tumours (e. g. ANDRES, 1932; DECKNER, 1938).

A very interesting observation was made by LEWIS (1935), who found that mitoses in tissue cultures treated with carcinogenic substances took place normally under dark conditions. In strong light, however, very considerable cytological disturbances were obtained. In some cases the nuclear spindle had totally disappeared; so the result was a c-mitosis-like process. The disappearance of the spindle was in this case probably due to a rather non-specific damage, similar to those damages induced on the spindle by strong X-ray dosages. A photosensitization of this kind is obtained thus: the activating substance has the ability to absorb the energy quanta of the light and transfer the energy to other molecules which are not able to make direct use of it.

Several other observations have been made of cells or organisms turning light-sensitive under the influence of carcinogenic substances. GRAFFI (1940 a) found that *Daphnia* could live for a long time in a benzpyrene solution in darkness, but exposed to ultraviolet light it was killed in 2—3 seconds. There are similar reports respecting other organisms, as *Paramaecium* (MOTTRAM and DONIACH, 1938) and yeast (HOLLAENDER, COLE, and BRACKETT, 1939). DONIACH and MOTTRAM (1940) brought about haemolysis of erythrocytes by illumination in a benzpyrene solution. It may be mentioned, although in this case the substance concerned is not a carcinogenic agent, that a very interesting biological photosensitization by eosin was obtained by DÖRING (cf. STUBBE, 1940) in *Neurospora*. The genes became sensitive to visible light and mutated after treatment of the cells with daylight illumination.

One type of cytological deviation, often come across both in spontaneous and induced cancer tissue, is the occurrence of polyploid cells. Usually tetraploid cells or cells with chromosome numbers around the tetraploid number are found, but also ± octoploid numbers occur. The occurrence of polyploid cells in cancer tissue was especially pointed out by WINGE (1928, 1930). He was lead to the hypothesis

that the great growth energy of cancer was caused by polyploidy. This hypothesis seems at present to be but little probable. It is founded on the over-estimation of the vigour of the polyploids that was earlier in vogue. Polyploids now produced in great numbers have turned out in most instances to be of inferior viability as compared with the original diploid material. It has also been directly shown in many cases that polyploid cells in diploid-polyploid chimaerical tissues as a rule have lower growth energy than the diploid cells, a fact that enables the latter to win the competition in most cases.

Chromosome-doubled cells occur frequently, however, in cancer tissue or tissue treated with carcinogenic agents, and their mode of formation will now be briefly inspected. In a few cases, in which some information about the mechanism of origin of the tetraploid cells has been gathered, it has been established that this does not agree with the c-mitotic mechanism. There are instead some indications that the mechanism is an endomitosis, i. e. a double reproduction of the chromosomes during the resting stage.

Some authors, studying the effect of carcinogenic agents, have reported the occurrence of meiosis-like processes in somatic cells, e. g. HEARNE (1936) and HEARNE CREECH (1939) in tissue cultures treated with carcinogenic hydrocarbons, and AMANO and ANDO (1940) in the liver of animals treated with o-amino-azotoluene. Diakinesis-like figures were seen with paired chromosomes held together by chiasmata. In such cases the occurrence of a c-mitotic mechanism might possibly be suspected, since the c-pairs, especially of long chromosomes, at certain developmental stages have a striking resemblance to bivalents. There is, however, another more probable mechanism for the explanation of these meiotic figures, viz. that demonstrated in the mutant *cancroidea* of *Antirrhinum*. STEIN (1936) initially thought she had observed a chromosome pairing in somatic cells. Later investigations, however, (1942) proved that these configurations were produced through an endomitosis which often was not completed, but interrupted at a more or less advanced stage by an ordinary mitosis. The arrangement of the chromosomes in pairs was produced by a double reproduction, as in *Spinacia* (GENTCHEFF and GUSTAFSSON, 1939). During the prophase she observed the lengthwise chromosome split earlier than normally. Our view that the reaction of the cells to carcinogenic hydrocarbons has been of the same kind is considerably strengthened by the observations of HEARNE CREECH (1939) that it is a characteristic feature of the chromosomes under this influence that the prophasic split is visible earlier than usually (»precocious splitting», »... the split halves fall apart into distinct nodes and loops resembling a meiotic prophase . . .»). WINGE (1928), studying phytocarcinomata of *Beta*, also observed octoploid cells of a diakinesis-like appearance with the chromosomes arranged in pairs. He supposed the mechanism to be a double reproduction. BROCK, DRUCKREY, and HAMPERL (1940) and LANGER (1942), studying the effect of 4-dimethyl-amino-azo-benzene on the liver, report as the first visible characteristic feature the occurrence of a nuclear polymorphism, especially an occurrence of giant nuclei. As the mechanism of origin of these giant nuclei, they suggest »innere Kernteilung».

Perhaps some of the giant cells produced through treatment with carcinogenic substances (yeast, *Paramaecium*, tissue cultures) may have become polyploid through endomitosis. Polyploidy through endomitosis was suggested as a possibility for the production of giant races of yeast through camphor treatment by

LEVAN and SANDWALL (1943). In the case of the endomitosis induced by phytohormones LEVAN (1939) arrived at the conclusion that the process was induced secondarily by the enlargment in cell size. The mechanism, of course, may or may not be identical in the case of the carcinogenic substances.

A feature characteristic both of the c-mitotic and the carcinogenic substances is their ability in some cases to inhibit growth, in other cases to stimulate growth.

An inhibition of tumour growth brought about by colchicine is known both from animal material (e. g. AMOROSO, 1935; SEED, SLAUGHTER, and LIMARZI, 1940) and plant material (e. g. HAVAS, 1939 b; DERMEN and BROWN, 1940). Carcinogenic substances have also been found to inhibit the growth of animal tumours or of whole animals (e. g. HAVAS and GAL, 1938; WHITE and WHITE, 1939; HADDOW and ROBINSON, 1939; MORELLI, 1941), or growth within tissue cultures (EARLE and VOEGTLIN, 1938) and in plant tissue (PATTON and NEBEL, 1940).

A growth-stimulating effect after treatment with c-mitotic substances is a phenomenon often observed. This effect usually appears after the plant has recovered from the growth retardation first produced. Instances of such stimulation are mentioned by NEBEL and RUTTLE (1938 b): »Growth after the stunting in some cases appeared more vigorous than in the checks». Such a stimulation after an initial retardation was also observed after acenaphthene treatment in *Lactuca* by KOSTOFF (1938 e) and in *Anthoxanthum* by ÖSTERGREN (unpublished). MÜNTZING and RUNQUIST (1939) obtained a stimulation after colchicine treatment in different plants, especially *Festuca* and *Lolium* (but they give, in contrast to the other instances cited, no report of an initial growth retardation). The unpublished results of JENSEN in this direction have been mentioned earlier. LEVAN (1940 b) found a weakly stimulating effect on the germination of red clover by a 0,001 % colchicine solution. A stimulation of the growth of micro-organisms by colchicine has been demonstrated by OBATON (1939) in *Photobacterium* and by RICHARDS (1938) in yeast.

Growth stimulation by carcinogenic stubstances has been reported within many widely different materials: *Paramaecium* (MOTTRAM, 1939), *Obelia* (HAMMET and REIMANN, 1935), and *Planaria* (OWEN, WEISS, and PRINCE, 1939). A growth-promoting effect in tissue cultures is described by HEARNE CREECH (1939). According to SHEAR (1937), a perilous use of the carcinogenic azo-compound scarlet red has been made clinically, viz. for stimulating the healing of wounds. Also within botanical material numerous cases of growth stimulation by carcinogenic substances are known: in Bacteria (GOLDSTEIN, 1937), yeast (DODGE and DODGE, 1937; HOLLAENDER, COLE, and BRACKETT, 1939; COOK, HART, and JOLY, 1939; TOLMATCHEWA, 1940), various higher plants (SHMUCK, GUSSEVA, and ILJIN, 1939); *Pisum* (OWEN, WEISS, and PRINCE, 1939) and *Ricinus* (CARMINATI, 1940). HAVAS (1939 b) mentions the stimulating of plant tumours, and KISSER and LINDENBERG (1940) in extensive experiments observed stimulation of root formation and wound healing in plants.

It is evident that in these various cases of inhibition and stimulation *many different processes may be concerned.* This fact may in certain cases yield an explanation of the contradictory results, in which the same substance has acted both inhibiting and stimulating. Thus, evidently the concentration of the substance is of decisive importance. It is understandable that if c-mitotic substances are employed in concentrations above their threshold value for c-mitotic action, the immediate effect will be an inhibition in growth because of the arrest of the mitoses. The fact that colchicine has been found to counteract the growth of plant and

animal tumours may be due to this. DERMEN and BROWN (1940) assume the inhibition of the tumour growth in plants to depend on the checking of the mitoses by the colchicine, which induces an »excessive polyploidy» in the tissue. This may evidently reduce the growth power of the tumour even after the end of the treatment. In other cases the inhibitory effects of c-mitotic and carcinogenic substances may be due to a purely non-specific poison effect.

The stimulative effect of the c-mitotic substances manifests itself partly immediately after the treatment with very low concentrations, partly some time after the treatment with higher concentrations. The former effect may be a direct hormonic action. The phytohormones act growth-promoting only in very low concentrations, while higher concentrations act growth-inhibiting. It may also be possible, as HAVAS (1938) suggests, that the stimulation acts through an activating of the hormones present in the plant.

The other type of stimulation appearing some time after the treatment may of course be of a similar kind to that following treatment with weak solutions. A certain time interval will have to elapse in order to bring the content of the substance within the tissues down to the suitable concentration range. In several of the cases mentioned the stimulation is active during such a long time after the treatment, in some cases even through several vegetative generations (JENSEN), that the impression comes very readily that it may be a question of a dauermodification. Another possibility has been suggested both for colchicine (RICHARDS, 1938) and for carcinogenic substances (PATTON and NEBEL, 1940), viz. that the organism has the power of utilizing the substances as food. Especially in the latter case, however, this suggestion does not seem very probable.

SUMMARY.

(1) The α-derivatives of naphthalene show a positive correlation between water solubility and threshold of c-mitotic action. (That is to say, their activity increases when the solubility decreases.)

(2) C-tumour formation is not caused by polyploidy, as it often shows some autonomy with regard to c-mitosis, for instance, a different threshold concentration.

(3) There are clear indications that the β-derivatives of naphthalene, too, are active. They differ from the α-derivatives by being active only within a much smaller concentration range.

(4) Ethyl alcohol is antagonistic to colchicine.

(5) A comparison between the effect of vapour and solution of acenaphthene indicates that, as compared with water, moist air counteracts c-mitosis but promotes c-tumour formation.

(6) Leguminosae (*Pisum*) are not immune to acenaphthene and naphthalene derivatives, but are less sensitive than *Allium*. To colchicine *Pisum* is, on the contrary, more sensitive than *Allium*.

(7) The practical efficiency of c-mitotic substances in producing polyploids is not due to their activity as measured by their threshold of action. Important factors for the practical purpose are the poisonousness of the substance and the relative size of the effective concentration range.

(8) The activity of the c-mitotic substances is due not to their chemical properties but chiefly or exclusively to their physical properties, just as is the case with narcotics. (There may, of course, be exceptions to this rule.)

(9) The MEYER—OVERTON theory of narcosis may also hold in the case of c-mitotic substances, i. e. the decisive concentration of the substance may be that in the lipoids, not that in the water phase of the cells.

(10) Alternatively it is possible that the primary feature in c-mitotic action is a condensing of lipoid coacervates, a phenomenon of the type studied by BUNGENBERG DE JONG. That would explain the antagonistic effect of ethyl alcohol.

(11) C-mitosis is similar to narcosis not only in being due to a physical action on the cell. It is, just like narcosis, a reversible disconnecting of some life-processes, while others go on practically normally. C-mitosis may be a kind of narcosis, as also may be the case with c-tumour formation.

(12) The question is discussed where in the cell the c-mitotic substances produce their action. Mitochondria or centromeres are suggested.

(13) Probably the similarity sometimes suggested between c-mitotic substances and carcinogenic substances also is due to a similarity in physical properties.

(14) There are some indications that c-mitotic substances and carcinogenic substances may induce dauermodifications.

(15) It is suggested that cancer may arise as a cell change of the dauermodification type.

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ELASTIC CHROMOSOME REPULSIONS

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THE forces controlling the appearance and behaviour of chromosome configurations have been discussed nearly as long as mitosis has been known. The older hypotheses on this subject are reviewed by TISCHLER (1922, pp. 333—348). The chief problem under discussion was usually the causes of chromosome movements. A more recent system of hypotheses on this subject is that given by DARLINGTON (1937, Ch. XII, pp. 479—562). He discusses not only the forces of chromosome movements but also the causal mechanism underlying the characteristic appearance of bivalents, as, for instance, the mechanism of chiasma terminalisation, etc.

The importance of the electrostatic forces suggested by DARLINGTON and others has no doubt been overestimated. Sufficient evidence can be brought forward to show that the electrostatic hypotheses of the shape of the mitotic figure and of chromosome movements are inadequate. Thus, the resemblance of the spindle fibres to electric or magnetic lines of force must be quite superficial. The equivalence of the poles in multipolar spindles shows that the spindle must be a homopolar structure, and in such a case the lines of force cannot proceed from pole to pole but must avoid each other. The equilibrium position of anisodiametric molecules or micellae should be either parallel or perpendicular to the line of force, depending on the kind of anisotropy characteristic of the particle. Neither type of equilibrium position would give a structure like that found in the spindle.

Even if simple electrostatic forces are of no great importance in that connexion they may still exist and have influence in »cell mechanics». Thus the repulsion between chromosomes and the nucleolus observed by UPCOTT (1936, p. 125) may find a simple explanation in this way.

It is not my intention here to discuss all theories of the »mechanism of mitosis» as, for instance, the traction fibre hypothesis, etc. Instead, my purpose is to call attention to a kind of force that influences the shape of chromosome configurations and that does not seem to have been noticed previously. I mean the repulsion between chromosomes

or chromatids due to simple elastic pressure at the points where they touch each other.

It is characteristic of all bodies which are not liquid (or gaseous) that they have a definite shape, and that efforts to change this shape meet with an elastic resistance. Deviations from this »ideal shape» mean an increase in the potential energy of the body, which increase the body will strive to remove again as soon as possible. The ideal shape of the metaphase chromatid seems to be a straight cylinder or rod with an even diameter (the spiralisation constant; DARLINGTON, 1937, p. 52), characteristic of each mitosis but modifiable from one mitosis to another by environment and genotype. [The exceptions to the constant spiralisation as, for instance, secondary constrictions and »differential segments» (DARLINGTON and LA COUR, 1940) have no great bearing on the discussion given below.] The uncoiling of the relational chromatid coiling during metaphase is caused by the efforts of the chromatids to reduce their potential energy by approaching their ideal shape. When metaphase is arrested by colchicine the chromatids have time to straighten completely and will now appear as straight rods.

MECHANICS OF THE COLCHICINE PAIR.

Under the influence of colchicine and many other substances (e. g. LEVAN and ÖSTERGREN, 1943) mitosis is highly modified. The chief factor in which the c-mitosis (LEVAN, 1938) differs from the normal mitosis is through the absence of the nuclear spindle. Because of this the chromosomes are arrested at a prolonged »metaphase». The chromosomes appear as very characteristic configurations, termed c-pairs by LEVAN (1938), who also gives a detailed description of them. A recent description of c-mitosis and c-pairs is given by MANGENOT (1942). This type of chromosome configuration has also been observed in animals (in a grasshopper) by SOKOLOW (1939).

In the c-pairs the chromatids uncoil their relational spiral and are finally directed like the arms of an X. The appearance strongly suggests the existence of a mutual repulsion between them, making them diverge as far as possible. The repulsion might be thought to be an electrostatic repulsion, of the type suggested by DARLINGTON to exist between chromosomes (body repulsions). I think, however, the chief factor is of another nature. The chromatids are held together by the centromere, which is (at least functionally) undivided and appears as a constriction. It is self-evident that there must be a mutual mechanical

pressure between the chromatids at this spot, a pressure against which they will offer an elastic resistance. The result is a repulsion between the chromatids localized at the centromere. This repulsion will make the chromatids diverge just as a body repulsion would. The mechanism is the same as that operating when a pair of rubber tubes are tied together (Fig. 1).

This hypothesis also provides a rational explanation of the development of the c-pair previous to the X stage. I cite from LEVAN (1938, p. 473): »At first loops are formed between the undivided centromeres and the points where, on account of the spiralisation tension, the chromatids touch each other. There usually occurs one such point on each of the long chromosome arms (Fig. 1 *i*—*p*). These points of contact move slowly towards the ends, and at last the chromatids touch each other only at the still undivided centromere and at one or both ends (Fig. 1 *n*, *o*; Fig. 2 *a*, *l*). This process strikingly resembles the terminalisation of chiasmata in diakinesis bivalents. At last the ends also slip off, and the half-chromosomes are now held together only at the undivided centromeres». This process, I think, strongly suggests that the force prizing the chromatids apart is localized closely proximal to the centromere. The end-to-end touching of the chromatids at a certain stage may perhaps even suggest that there is a slight stickiness between the chromatids, which has to be overcome by the proximal repulsion.

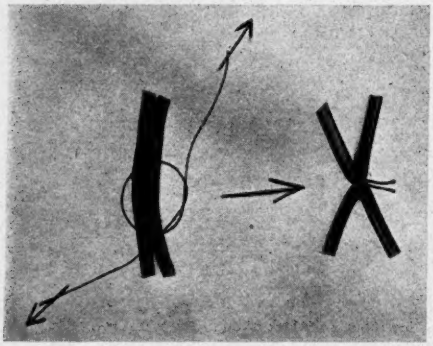

Fig. 1. Model experiment illustrating the mechanics of the c-pair. When a pair of rubber tubes are tied together, they will diverge because of the elastic repulsion at the point of contact.

When the centromeres of the c-pairs have divided, the two chromatids straighten and lie side by side like »pairs of skis» (NEBEL and RUTTLE, 1938, p. 4 and LEVAN, l. c., p. 474). Had there been any considerable body repulsion between them, they would probably not have remained so close together but would have moved further apart.

The proximal, elastic repulsion may perhaps also play a part in causing the transverse division (misdivision) of the centromere observed during c-mitosis by KARPECHENKO (1940). In a normal metaphase chromosome where the chromatids are coiled together the elastic pressure

between them at the centromere will be dircted in such a way that it highly favours the normal lengthwise splitting of this body, the pressure acting between sister chromatids and trying to separate them. When a cross-shaped figure has resulted during colchicine treatment, however, there will be a more or less equal pressure between all the four chromatid arms, i. e. the centromere is subjected to forces trying to split it not only longitudinally but also transversely.

CHIASMA MECHANICS.

To explain the characteristic appearance of bivalents during the meiotic prophase DARLINGTON and DARK (1932) and DARLINGTON (1937) assume the existence of two kinds of electrostatic repulsions, viz. a general body repulsion, due to the surface charge of the chromosomes and an especially strong centric repulsion between the centromeres, due to a localized charge of these bodies. The body repulsion is responsible for the opening out in loops of the bivalent between chiasmata, and also for the fact that successive loops lie in planes forming right angles to each other. The centromere repulsion gives the centric loop a characteristic shape, different from the other loops. It is also responsible for the movements of chiasmata away from the centromere, i. e. chiasma terminalisation.

Now a chiasma is a point where the chromosomes touch each other in a similar way as the chromatids do at the centromere in a c-pair. Consequently it is only natural to assume that there should be elastic repulsions at work here, too. Such repulsions, no doubt, may be responsible for the loop formation between chiasmata and also for the fact that the successive loops lie at right angles to each other.

Elastic chromosome repulsions may also play a part in chiasma terminalisation. This may be understood from Figs. 2 and 3. Fig. 2 is a bivalent held together by two chiasmata. To simplify a study of the conditions it is assumed to be acentric. At the right chiasma there is an elastic repulsion both in the angle α and in the angle β. The pressure will be greater in α than in β because of the bending of the chromosomes on the left of α. This means that the potential energy of the angle α is greater than that of β. If the chiasma is terminalized (decrease of the distance D) the potential energy of α will decrease and that of β will be practically unchanged until the chiasma has moved so far to the right that the surfaces touching each other at β begin to decrease in size. Then the potential energy of β, too, will decrease.

Approximate curves of these relations are given in Fig. 3. Thus the potential energy of the chiasma as a whole will decrease when it is moving towards the right. As all systems tend to decrease their potential energy it can be predicted that the chiasma will terminalize. This may be the background of the phenomenon mentioned by DARLINGTON (1937, p. 514): »Closed loops repel one another more than open arms: for distal chiasmata move towards the ends even though no other movement is detectable».

Fig. 2. An acentric bivalent with two chiasmata.

It is evident that these considerations cannot alone account for the terminalisation of chiasmata. The elastic repulsion is just one of many factors controlling chiasma terminalisation. Thus the centric repulsion, as suggested by DARLINGTON, must, of course, play a very

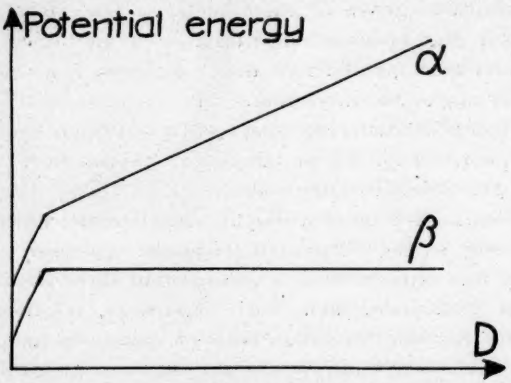

Fig. 3. Approximate diagram of the potential energy of the angles α and β of Fig. 2, showing the variations to be expected following changes in D. (The curves illustrate the general principles of the connexion. They are not meant to be exact in details.)

important rôle in terminalisation. It is possible, however, that this repulsion is not electrostatic in nature. A general body repulsion due to the surface charge of the chromosomes may of course also play a rôle in chiasma mechanics in a way similar to that pointed out by DARLINGTON.

SPACING OF CHROMOSOMES IN THE NUCLEUS.

During prophase stages the chromosomes are as a rule evenly spaced within the nucleus. This has usually been ascribed to their

mutual electrostatic repulsions (as in the text-book by DARLINGTON, 1937, p. 503). Another factor which undoubtedly may contribute to their spacing is the fact that during the preceding resting stage they had a much larger volume. In many cases, in their much hydrated and dispersed condition, they filled up practically the entire volume of the nucleus. When they decrease their volume at prophase, through dehydration and spiralisation, they will most probably come to lie at the approximate centre of their previous volume. From this it follows that they will lie well separated.

SUMMARY.

The importance of the mutual elastic pressure between chromatids or chromosomes at their points of mutual contact is pointed out. This pressure gives a simple explanation of the repulsion between the chromatids of the c-pair, touching and pressing at each other at the undivided centromere. From this localisation of the repulsion the characteristic end-to-end touching of the chromatids at a certain stage of c-pair development is understood. These forces may play a rôle at the misdivision of the centromere at colchicine treatment observed by KARPECHENKO. They are probably also operative at the chiasmata of bivalents, and will contribute to bring about chiasma terminalisation, and to give bivalents their characteristic shape. The spacing of chromosomes in the prophase nuclei may be due to their much greater volume during the preceding resting stage. When they contract, they will be lying at the approximate centre of their previous volume.

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SPONTANEOUS CHANGES IN CHROMOSOME NUMBER IN APOMICTIC POTENTILLA COLLINA

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The species *Potentilla collina* belongs to a number of *Potentilla* species which were found to be apomictic (MÜNTZING, 1928, 1931, 1941). Pollination is necessary for seed development, but the egg cells are not fertilized. The offspring are strictly maternal and completely uniform. This behaviour was observed in seven different biotypes of *P. collina.* One of these biotypes, *C—A* (derived from the island of Gotland), was pollinated with pollen from different biotypes of *P. Tabernaemontani, Crantzii* and *argentea.* From the seeds produced a total of 25 plants were obtained, representing four different cross combinations (MÜNTZING, 1928, Tab. 6). As in all other crosses with *P. collina* as the female parent, the 25 daughter plants of *C—A* were all maternal, thus indicating a completely apomictic mode of seed formation. Absolute apomixis in *C—A* was further indicated by the complete constancy in progenies raised from isolated or openpollinated mother plants. This observation of complete constancy in *C—A* was based on three different progenies comprising a total of 120 individuals.

In 1939 most of the *Potentilla* material was transferred from Svalöf to Lund, and a number of new progenies were raised. A new progeny of *C—A* comprised a total of 20 plants. At first sight this progeny appeared to be quite uniform as usual, but a closer inspection revealed the presence of a somewhat deviating individual. This plant (»117—6») had certain gigas properties, the stems being thicker and the flowers larger than in the normal sister plants (cf. Fig. 1). It was also found to have much better pollen fertility than the sister plants. In the gigas plant the average percentage of good pollen was 85. In five normal sister plants this percentage ranged from 40 to 60 with an average of 50,0. On studying meiosis in the p. m. c. of the gigas plant this plant was found to be tetraploid in relation to normal *C—A*, the chromosome numbers being $2n = 84$ and $2n = 42$ respectively. The latter number had been established previously (MÜNTZING, 1931, Table 1).

For further study new progenies were raised from the gigas plant as well as from a normal sister plant. The former progeny (1941—15) was raised after isolation, the latter one (1941—14) after open pollination. The progeny 1941—15 comprised a total of 33 plants, the progeny 1941—14 consisted of 38 plants. In the first place counts of the somatic chromosome numbers were undertaken in this material. In the normal *C—A* family all 38 plants were examined. Of these individuals 37 were found to have $2n = 42$ or ± 42, but one plant had $2n = \pm 84$. The latter plant seems to represent a second case of chromosome doubling in *C—A*.

In the other progeny, 1941—15, the results of the chromosome counts were still more puzzling. Unfortunately, root tips were only fixed from ten of the plants. In eight of these individuals the chromosome number was 84 or $\pm$ 84 as expected, but the remaining two plants had $2n = 42$ and $2n = 43$ respectively. Thus, judging from these results, »tetraploid» *C—A* is not quite stable in chromosome number but has a tendency to revert to the normal number, $2n = 42$.

As the progenies of »diploid» and »tetraploid» *C—A* were raised at the same time, the appearance of tetraploids in the former progeny and of diploids in the latter one might be suspected to be due only to experimental errors. If by mistake a few seedlings of No. 14 had been put in the box containing No. 15, and vice versa, this might explain the whole variation. Even though such experimental errors may possibly account for part of the variation in 1941—14 and 1941—15, it is now perfectly clear that *in C—A there is really a variation in chromosome number from 42 to 84 and from 84 back to 42 or approximately this number.* The change from 42 to 84 is evidently a rather rare phenomenon, and it cannot yet be stated with certainty that this chromosome doubling has occurred more than a single time, producing the plant »117—6». On the contrary, the reversion from 84 to approximately half the number is evidently a more frequent phenomenon. This is evident from the following facts:

(a) Of the two plants with low chromosome numbers in the progeny 1941—15 one had $2n = 42$ and was a typical *C—A* plant. The second plant, on the contrary, had $2n = 43$, as was ascertained by repeated counts of somatic chromosome plates from different slides. This plant was also morphologically deviating and differed from typical *C—A* by a somewhat reduced vigour, smaller flowers of a more pale yellow colour, smaller and more narrow leaves and by having rather defective anthers. In typical *C—A* the number of petals is always 5, but in the

plant with 2n = 43 a rather high proportion of the flowers had only 4 petals. As ordinary *C—A* plants (excepting the rare occurrence of gigas plants with doubled chromosome number) always have 2n = 42 and do not vary morphologically, the deviating plant with 43 chromosomes cannot represent an intermixture but must be a true daughter plant of the 84-chromosome plant.

As mentioned above, only 10 of the 33 plants in the progeny 1941—15 were examined as to the somatic chromosome number. Of the remaining 23 plants 11 were typical gigas plants, evidently having 2n = 84 like the mother plants, 7 plants were of quite the same appearance as normal *C—A* (2n = 42) and 5 plants, finally, were new, more or less aberrant types. Like the 43-chromosome plant described above, these morphologically deviating plants, at least, must be true daughters of the 84-chromosome mother plant. From studies of meiosis one of them was later on found to have ± 42 chromosomes.

(b) In 1942 a new progeny of the primary plant with 2n = 84 was raised. This time care was taken not to germinate normal *C—A* at the same time. In fact, the progeny raised was the only representative of *Potentilla collina* this year. Thus, intermixture with normal *C—A* or other *collina* types is entirely excluded. A total of 49 plants were raised, and in all these plants the somatic chromosome numbers were determined. In 44 of the plants the number was found to be 84 or ± 84, but in addition to these there was again a minority of plants having about half of this chromosome number. In the present case this minority consisted of 5 plants having 2n = 42 (2 plants), 2n = ± 42 (1 plant), 2n = 40 (1 plant), and 2n = 39 (1 plant). Thus, the production of a regular proportion of polyhaploids in the offspring of »tetraploid» *C—A* is absolutely certain.

In the present progeny the frequency of such plants with 2n = ± 42 is 10 per cent. In the previous progeny at least 7 and possibly as many as 14 plants of 33 were polyhaploids. This would correspond to a minimum percentage of 21 and a maximum percentage of 42. Further information about the true frequency will be obtained from a morphological study of the new polyhaploids, which are not yet fullgrown. Already at an early stage, however, they could be distinguished at first sight from the plants having 2n = ± 84.

Before leaving the *C—A* material it may be mentioned that with regard to total vigour the plants with 84 chromosomes were clearly inferior to the normal *C—A* plants having 2n = 42. It is possible, though, that this difference may be due to a slower rate of development in the 84-

chromosome plants and that these plants in later years may increase in relative vigour. At present, however, the gigas properties of these plants are limited to thicker stems and leaves, and larger flowers and seeds. The flower diameter was measured with the following result.

	Flower diameter in mm.										
	12	13	14	15	16	17	18	19	20	n	M ± m
1941—14 (*C—A*, 2n = 42)			9	13	10	4				36	$14{,}_{75} \pm 0{,}_{16}$
—15 (» , 2n = ± 84)					3	8	6	1	1	19	$16{,}_{92} \pm 0{,}_{23}$
—15 (» , reversions)	2	6	3	2						13	$13{,}_{88} \pm 0{,}_{27}$

The above values demonstrate a significant difference in flower size between normal *C—A* and its tetraploid derivative. This difference is also evident from Fig. 1. Of more interest, however, is the fact that

Fig. 1. Flowers of *Potentilla collina, C—A.* Upper row: 2n = 42, lower row: 2n = 84.

the reverted *C—A* plants having ± 42 chromosomes have a smaller average than the normal *C—A* plants. The difference is $0{,}_{87} \pm 0{,}_{31}$, giving a t of 2,81 and a P intermediate between 0,01 and 0,001. The flower average value of the reversions is certainly due to the fact that this category includes some aneuploid individuals. The depressing influence of aneuploidy is probably also responsible for the somewhat lower pollen fertility in this category than in normal *C—A* (cf. p. 458).

It should be mentioned that single plants with a doubled chromosome number were also observed in two other, morphologically different, progenies of *P. collina*, grown at the same time as the *C—A* material. These progenies, 1941—63 and -64 were derived from openpollinated mother plants collected at two different localities on the island of Öland (Vickleby and Resmo). The former progeny consisted of 39, the latter

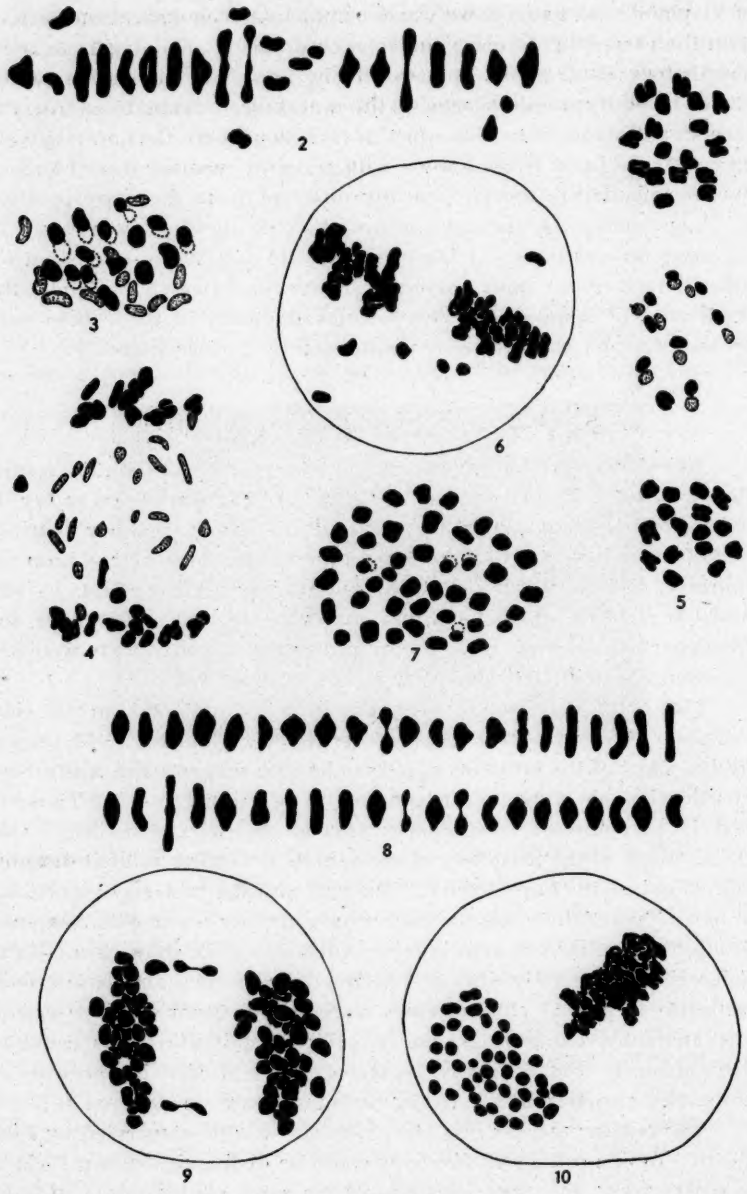
of 21 plants. As usual there was a complete morphological uniformity with the exception of one plant in each progeny. Judging from their morphology these plants appeared to be tetraploid in relation to the other plants. By studies of meiosis this was, indeed, found to be true, the exceptional plants in both families having $2n = \pm 84$, the normal plants $2n = 42$. So far it is not known with certainty whether these 84-chromosome plants are morphologically different from the corresponding *C—A* products. As, further, the progenies in question were raised at the same time as the *C—A* families 1941—14 and 1941—15, risk of an intermixture is not quite excluded. Therefore, though probable, the occurrence of spontaneous chromosome doubling in the *collina* progenies 1941—63 and -64 needs verification by further studies.

MEIOSIS AND FERTILITY.

Meiosis was studied in normal and tetraploid *C—A* with the results shown by Figs. 2—10. In normal *C—A* ($2n = 42$) meiosis is rather irregular owing to an incomplete pairing at I—M and irregular distribution of the univalents (Figs. 2—6). In the 84-chromosome *C—A*, on the contrary, meiosis was found to be remarkably regular (Figs. 7—10). At I—M most or all of the chromosomes are present as bivalents, the frequency of non-conjunction being rather low. Multivalents were not observed either in »tetraploid» or in normal *C—A*.

Figs. 2—3 represent two I—M groups of normal *C—A* (in side view and polar view) with the configurations $16_{II} + 10_{I}$ and $15_{II} + 12_{I}$ respectively. Most of the bivalents appear to be ring-shaped. Fig. 4 is a I—A in side view showing the distribution $14 - 1 + 20/2 - 17$. Thus, 14 and 17 chromosomes respectively pass to the poles undivided. One chromosome at the periphery of the spindle is lagging without division, and 10 univalents have divided. The half univalents clearly correspond in pairs. Two of these half univalents have already reached the anaphase groups. A similar situation is represented by Fig. 5, showing a I—A in polar view (separately drawn). In this case the two anaphase groups comprise 16 and 17 chromosomes respectively. Between these groups one undivided chromosome and 8 pairs of split univalents could be distinguished. Fig. 6, finally, represents a II—M, showing presence of eliminated chromosomes. Lagging chromosomes were observed at II—A.

The regular meiosis of »tetraploid» *C—A* is demonstrated by Figs. 7—10. In one cell all 42 bivalents could be distinguished in a I—M in side view (Fig. 8). Fig. 7 represents the same configuration in polar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view. Fig. 9 shows a I—A with two lagging and dividing univalents, and Fig. 10 represents a II—M with 42 chromosomes in the visible plate.

Further information as to the mode of meiosis may be gathered from the following estimations of the number of univalents present at I—M (observed in side view). In this table counts from the *collina* biotypes 1941—63 and -64 may also be included. As was mentioned above, both these biotypes have 2n = 42 like normal *C—A*.

	Number of univalents at I—M																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	n	M
C—A (2n = 42):					1	6	2	3	4	3	4	4	1	1		29	8,10
» (2n = 84):	24	10	12	2	2											50	0,96
1941—64:	14	6	18	5	6	1										50	1,72
—63:				2	2	4	5	4	5	1	1	1	3	1	1	30	7,50

From the above values it is evident that the frequency of univalents is quite considerable in *C—A* (2n = 42) and very low in *C—A* (2n = 84), especially when the difference in absolute chromosome number is taken into consideration. In the biotype 1941—64 the frequency of univalents is about as high as in *C—A* (2n = 42), but in 1941—63 it is much lower.

In the two *C—A* types the frequency of univalents dividing at I—A was also counted. In 50 p. m. c. of *C—A* (2n = 84) this number was found to range from 0 to 4 with an average of 0,76. In normal *C—A* (2n = 42) only nine cells at this stage were available. In these cells the number of dividing univalents ranged from 2 to 11 with an average value of 7,33. The values obtained evidently correspond very well to the number of univalents present at I—M (0,96 and 8,10 respectively). They also indicate that a low proportion of the univalents pass to the poles without division.

Pollen fertility was studied in the same material, the percentage of good pollen showing the following variation (see p. 458).

A comparison between these values and the univalent frequencies reveals a pronounced negative correlation between univalent frequency and pollen fertility. In the types with the most regular meiosis, *C—A* (2n = 84) and 1941—64, the percentage of good pollen was as

Figs. 2—10. Meiosis in *C—A* plants with 2n = 42 (Figs. 2—6) and 2n = 84 (Figs. 7—10). — Fig. 2, I—M in side view (separately drawn), $16_{II} + 10_{I}$; Fig. 3, I—M in polar view, $15_{II} + 12_{I}$; Fig. 4, I—A in side view, distribution 14—1 + 20/2—17; Fig. 5, I—A in polar view, distribution 16—1+16/2—17; Fig. 6, II—M with several chromosomes outside the II—M plates. — Fig. 7, I—M in polar view, 42_{II}; Fig. 8, I—M in side view (separately drawn), 42_{II}; Fig. 9, I—A, 2 univalents lagging and dividing; Fig. 10, II—M with 42 chromosomes in the visible plate. — × 4200.

	Per cent good pollen											n	M
	0—10	—20	—30	—40	—50	—60	—70	—80	—90	—100			
1941—14 (*C—A*, 2n = 42):			5	7	6	2						20	37,5
—15 (*C—A*, 2n = 84):							2	2	13	2		19	82,9
—15 (*C—A*, reversions to 2n = ± 42):			5	7	1							13	31,9
1941—63:	2	11	7									20	17,5
—64:					1	1	9	9				20	68,0

high as 82,9 and 68,0 respectively. The contrary was the case in normal *C—A* (2n = 42) and in 1941—63, having a high frequency of univalents, the percentages of good pollen being as low as 37,5 and 17,5 respectively.

DISCUSSION.

The main result of the present investigation is the demonstration that spontaneous chromosome doubling may occur in apomicts of *Potentilla collina*, and that a rather high proportion of the offspring of the resulting tetraploids revert to about half the chromosome number of the mother plant. At present the mechanism underlying the sudden chromosome doubling is entirely unknown. The most plausible assumption is the occasional formation of a tetraploid embryo-sac (having 2n = 84) and apomictic embryo development without fertilization of the egg cell. If the tetraploid arose by fertilization of the egg cell in an ordinary aposporous embryo-sac, the fertilizing pollen grain must also have been unreduced. The formation of some unreduced pollen grains is not excluded, and in one of the *collina* biotypes studied (1941—63) a single p. m. c. with twice the normal chromosome number was, indeed, observed. However, if fertilization takes place, the result would be, in the first place, »triploid» plants with 2n = 63. Such plants have not been observed to occur. As, further, normal *C—A* seems to be completely apomictic, the first alternative mentioned, the formation of a tetraploid embryo-sac (or a small somatic sector with doubled chromosome number) is the most plausible one.

The mode of propagation of tetraploid *C—A* (2n = 84) is in part elucidated by the rather frequent formation of daughter plants with chromosome numbers ranging from 39 to 43. These plants must have originated from reduced embryo-sacs, thus demonstrating a rather pronounced sexual tendency. However, probably all the egg cells, reduced as well as unreduced, develop parthenogenetically, without fertilization. So far, no true variation in morphology or in chromosome number has

been observed among the tetraploid plants. They all appear to be identical and, thus, most probably they have arisen parthenogenetically from unreduced aposporous embryo-sacs. The slight but significant variation in chromosome number in the offspring derived from reduced embryo-sacs is certainly due to slight meiotic irregularities. Such irregularities were observed in the pollen (Fig. 9) and may be expected to occur on the female side as well.

It would be quite important to know whether normal *C—A* (2n = 42) has the same embryology as tetraploid *C—A* (2n = 84). In such a case »haploid» embryos with 2n = ± 21 would be formed. As already pointed out, *C—A* is quite constant (excepting the rare production of plants with 2n = 84), and haploid aberrants do not occur in the offspring. It is possible, however, that haploid embryos are formed but that they die at an early stage. This possibility is supported by the meiotic studies in the p. m. c. Meiosis in normal *C—A* was rather irregular, being characterized by a high frequency of univalents. Tetraploid *C—A*, on the contrary, had a very regular meiosis with bivalents, very few univalents and no multivalents. This meiotic behaviour as well as the fertility conditions strongly indicate that normal *C—A* has a hybrid constitution. Haploids from such a hybrid would not be viable.

The only other possibility of explaining the production of haploids in the offspring of 84-chromosome *C—A* in contrast to 42-chromosome *C—A* is the assumption that the former type has a stronger sexual tendency than normal *C—A*, i.e. a stronger tendency to the formation of functional reduced embryo-sacs. Embryological investigations, now under way by Dr. ARTUR HÅKANSSON, may decide which of the two alternatives is the correct one.

Embryological studies of some other *collina* types in our material have already been made by GENTCHEFF and GUSTAFSSON (1940). The main result of their interesting paper is the demonstration that parthenogenesis in *Potentilla collina* and *argentea* is autonomous, but that endosperm and mature seeds can only be produced by a fertilization of the central nucleus. Information is given about the embryology of four different *collina* biotypes. Unfortunately the *C—A* biotype was not included in this material. The material studied was characterized by a more or less pronounced degree of polyembryony, the embryos generally arising in separate embryo-sacs. Of these embryo-sacs the one derived from the true or axile E.M.C. is probably always the result of a meiotic development, the lateral E.M.C's developing more or less mitotically. This indicates that reduced embryo-sacs may also be formed in normal

collina types. Whether they are also capable of embryo development remains to be decided.

The reversion in chromosome number from 84 to ± 42 in *Potentilla collina* is rather similar to the behaviour of certain hybrids between sexual and apomictic strains of *Poa alpina* (MÜNTZING, 1940, p. 153). In the offspring of such hybrids with 2n = 41 the majority of the plants had chromosome numbers ranging from 35 to 44, but in addition to these about 15 per cent of the plants were haploid with chromosome numbers ranging from 19 to 23. This must be due to a combination of the parental tendencies to chromosome reduction and parthenogenetic development. This material was studied embryologically by HÅKANSSON (1943). He finds that the egg cells often divide early, and that if no pollination occurs presumably all egg cells divide. The moment at which the pollen tube enters the embryo-sac decides whether a haploid or a diploid embryo will be formed. In any case, however, the polar nuclei must be fertilized.

The present case in *Potentilla collina* differs from that in *Poa* in as much as no fertilization at all seems to occur. The offspring consist of two groups derived from unreduced or reduced embryo-sacs respectively. In contrast to *Poa* the first group is quite uniform and constant in chromosome number, but the second group, just as in *Poa*, is somewhat variable owing to meiotic irregularities. In *Poa* the tendency to fertilization was derived from the sexual parent. In *Potentilla* neither the original type nor the tetraploid derivative has any tendency to fertilization of the egg cells.

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MEIOSIS IN A HYBRID WITH ONE SET OF LARGE AND ONE SET OF SMALL CHROMOSOMES

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IN his monograph on the North-American species of the genus *Godetia* HITCHCOCK (1930) has separated two subspecies or varieties of *G. Bottae*. He calls them *G. Bottae* var. *deflexa* and *G. Bottae* var. *typica*. The former is a rather robust and vigorous type, the latter a small and slender one. HIORTH (1941) considers that these types must be given the rank of species, *G. deflexa* and *G. Bottae* respectively, as already JEPSON had done. HIORTH has also produced the hybrid between them, which I have now examined. His *G. Bottae* (No. 6) agreed with var. *typica*; it was only 20 cm. tall, was richly branched and had small leaves. Of *G. deflexa* he had two types; No. 5 *a* was 1—1,3 m. tall, had few branches and large broad leaves, No. 5 *b* was barely 1 m. tall and had slightly narrower leaves than *a*. *Godetiae* cultivated under the name of *G. Bottae* are as a rule *G. deflexa* (l. c., p. 205).

G. deflexa and *Bottae* along with *cylindrica* form a special group of species, the *deflexa* group, the two last-mentioned species being most like each other (HIORTH, l. c.). They all have $n = 9$ chromosomes. A chromosomal dissimilarity has, however, been shown between *deflexa* and *Bottae* (HÅKANSSON, 1941). Whereas the robust species has large chromosomes, the slender one has small. The difference in size is considerable both as regards somatic chromosomes and bivalents. The somatic chromosomes of *Bottae* have so far only been studied in young petals. It is however evident that the *Bottae* chromosomes are only $^1/_3$—$^1/_4$ the length of *deflexa* chromosomes, and they also seem to be of a smaller diameter. The difference in the chromosome volume of the cells in the two species is therefore very great. *G. deflexa* also proved to have larger pmc's than *Bottae*, but the difference in cell volume is proportionately much less than the difference in chromosome volume. The size of the *G. deflexa* chromosomes has often been studied, in several hybrids as well (see CHITTENDEN, 1928; HÅKANSSON, 1943).

As regards *G. Bottae*, besides the No. 6 type, plants from a type Carmel in the neighbourhood of Monterey, Cal., have been examined. Small chromosomes also characterize *G. cylindrica*, which according to HIORTH is most like *Bottae* in habit. It may be added that among *Godetia* species hitherto examined several have the same chromosome size as *deflexa*, but that still more have the same size as *Bottae* (HÅKANSSON, 1943).

The meiosis of *G. deflexa* × *Bottae* was studied in plants 1319, 1320 and 1321 of S 987—1942. For the production of the hybrid, which is entirely sterile, *deflexa* 5 *a* had been used. It was an advantage that the meiosis was well fixed, since theoretically it is of rather great importance to examine a hybrid whose parents show a considerable difference in chromosome size. It is true that many hybrids between species having different chromosome size have been examined, but in general the difference in size has doubtless not been very great, in any case less than that in the hybridization under consideration. Recent investigations have now consistently shown that this difference disappears in the hybrid. Older investigations had, as known, given another result. »A distinction between the large chromosomes of one species and the uniformly smaller chromosomes of another species (a distinction such as could hardly be due to a structural change) was found to be retained in the hybrid. These descriptions have not been confirmed» (DARLINGTON, 1937, p. 56). Thus, whereas the result obtained in older investigations was that the chromosomes of the parent species remained unchanged in the hybrid, more recent investigations, among them several re-examinations of previously studied hybrids, have shown that this is not the case: the chromosomes of one or both parents have undergone change and the chromosome size is subjected to a genotypic control (DARLINGTON).

G. deflexa × *Bottae*, however, affords poor support for this view. The chromosomes are very clear in the pmc's after the dissolution of the nuclear membrane. There are nine small and nine large univalents, complete asyndesis existing in more than 50 % of the pmc's. The small chromosomes are not only shorter but also of less diameter than the large ones. The shape of the chromosomes could also be studied, as the region of the centromere showed up distinctly. The chromosome size seems to be unchanged in the hybrid, the volume of the *deflexa* chromosomes does not appear to have diminished, nor have the *Bottae* chromosomes increased in size or *vice versa*. Mitoses in young petals also showed nine large and nine small chromosomes. A somatic plate

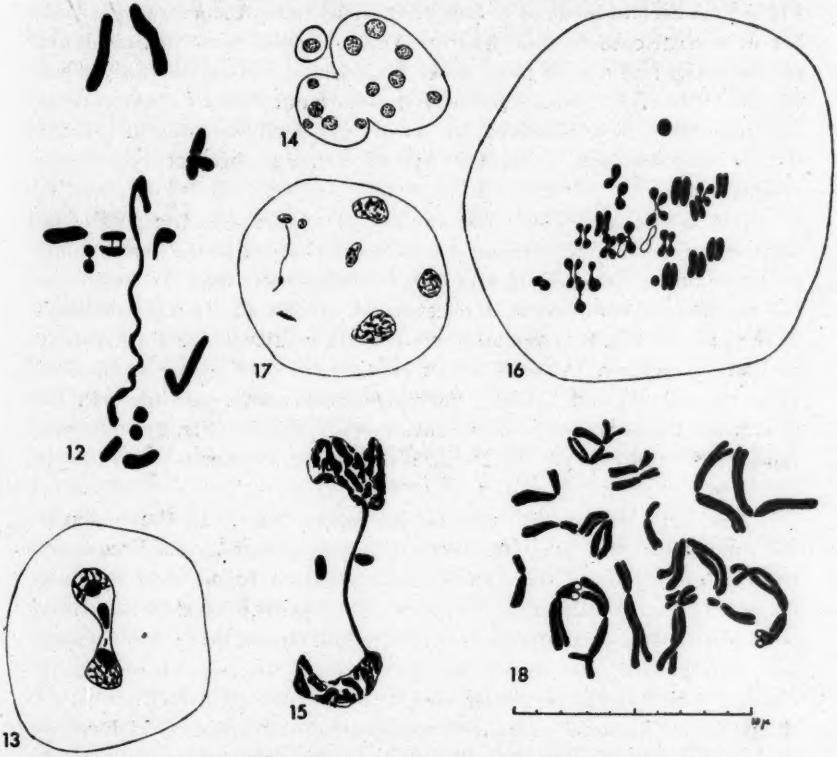
in a root tip is shown in Fig. 18. Without doubt the chromatids of the small chromosomes are considerably narrower than those of the large ones. Apparently the fusion of the parent nuclei was in a manner incomplete as eight of the small chromosomes lie together at one part of this plate. This is also rather interesting and calls for further investigations.

Meiotic prophase presents a rather mitotic picture. The nucleus contains long, slender threads, and the spiralization and consequent shortening of the threads during prophase is not so pronounced. The strepsitene stage is not observed, and the very considerable shortening of the chromosomes at the end of prophase (diakinesis) that is found in *deflexa* and in intraspecific *deflexa* hybrids (HÅKANSSON, 1943) does not occur. The chromosomes are therefore still very long when the nuclear membrane disappears (cf. Figs. 2 and 3). Chromosome pairing is evidently rare in occurrence. Strepsitene bivalents with paired chromosomes twisted round one another are not seen. Solitary bivalents probably occur, however, there being discernible threads that lie parallelly or, at the end of prophase, chromosomes that are attached to each other at one end. Only rarely, however, is this the case. The prophase nucleus contains a large nucleolus with which a chromosome is in contact. In addition there is a round body that does not consist of nucleolar material but of heterochromatin (verified by BHADURI staining). Not infrequently the nucleus has two large nucleoli side-by-side, which as a rule are of somewhat different sizes (Fig. 1). Between them was something that appeared to be a heterochromatic bivalent, which was in contact with both the nucleoli. It was impossible to decide whether this was a whole bivalent formed by two *Bottae* chromosomes or part of a bivalent. Its appearance seems to be associated with the formation of the two nucleoli, since the two phenomena regularly attend each other. Towards the end of prophase the chromatin threads are much thicker than in the stage illustrated in Fig. 1.

After the disappearance of the nuclear membrane the chromosomes may be very long. Solitary univalents appear to be less spiralized than the rest of the chromosomes (Figs. 2 and 3). As a rule, however, they are shortened. Many pmc's contain one or more bivalents. These only shorten in certain parts and therefore remain very long. The bivalents have a peculiar appearance (Figs. 5 and 8). They have a terminal or, very rarely, a subterminal chiasma (Figs. 8 and 10). Those chromosome arms between which the chiasma is formed are very long and only slightly spiralized, the other arms are normally spiralized. At

Figs. 1—11. *Godetia deflexa* × *Bottae*. — 1: part of a nucleus from a pmc. Two large nucleoli, a heterochromatic bivalent and a heterochromatic body are observed. — 2: metaphase 1, 2 I and 1 II (the latter to the right), very weak spiralization. — 3: early metaphase 1. — 4: early metaphase 1, 9 large and 9 small I. — 5: 2 heteromorphic II, a homomorphic small II, 7 large and 5 small I. — 6: early metaphase 1, 1 heteromorphic II, 8 large and 8 small I. — 7: no equatorial plate is formed, 18 I. — 8: 4 II, 2 abnormally long I with a longitudinal orientation in the spindle, 3 normal, large I and 5 small I. — 9: polar view of the equatorial plate, in the centre a II. — 10: a heteromorphic II with a subterminal chiasma. — 11: univalents with satellites. — × 3800.

the centromere there is often — especially in large chromosomes — a very sharp constriction. Thus, each paired chromosome has one very long and thin arm and one short and thick one. On account of the sharp constriction the latter arm frequently presents the appearance of



Figs. 12—18. *G. deflexa* × *Bottae*. — 12: anaphase 1, a despiralized I with its centromere at the equator of the spindle. × 3800. — 13: pmc with a restitution nucleus. × 875. — 14: restitution nucleus, newly formed. 7 large and 6 small nucleoli. × 2250. — 15: interkinesis, probably an inversion bridge. × 2250. — 16: early metaphase 2. × 2250. — 17: pmc after an irregular 2nd division. — × 875. — 18: somatic plate.

a free univalent in contact with the end of a bivalent or an elongated univalent. However, the above interpretation is the correct one. Very rarely was a bivalent seen presenting another appearance: in Fig. 6 are two chromosomes of different size that are lying a bit from each other but in such a manner as to suggest that two terminal chiasmata had

been formed. The bivalents are of three different kinds. Most of them are formed from a large and a small chromosome, i. e. by allosyndesis (Fig. 10, two bivalents in Fig. 5). Then there are small *Bottae* bivalents formed by autosyndesis from *Bottae* chromosomes (one bivalent in Fig. 5). A certain bivalent of this kind seems to be formed rather often, for in several cases a pmc had one small bivalent and 16 univalents, or one small and one or more other bivalents as well as the univalents. Finally, a bivalent formed by autosyndesis from *deflexa* chromosomes has sometimes been observed, an hardly expected observation because in the 4n *Whitneyi* × *deflexa* hybrid *deflexa* chromosomes never conjugated.

It is not easy to count the number of bivalents in the pmc's, the plate often containing elongated univalents that lie in the longitudinal direction of the spindle and that may be abnormally long. To determine the number of bivalents it is necessary to count all the chromosomes in the pmc's. Fig. 8 appeared to contain six bivalents, but there cannot be more than four. At least 50 per cent. of the pmc's have no bivalent (Figs. 4 and 7), and hence a very strong asyndesis prevails. In the next place the occurrence of one bivalent is common (Fig. 9). A larger number is rather rare and more than four bivalents were never observed.

The large univalents could be studied the best. In the region of the centromere they presented very different appearances. Frequently the two chromosome arms were separated by a broad zone that was colourless, i. e. unstained by gentian violet (Fig. 6). No structure could be detected with certainty in this zone. Sometimes there was no such intervening zone, the arms being joined by a thread instead (Fig. 7, centre), which could be rather long. If a thread connected the arms, they had an instable position to each other; the arms could have an almost parallel course. Finally, at the centromere there could be a constriction. The extent to which the fixing agent contributed to the production of these structures may be left an open question. In two of the large univalents the arms were of the same length, in two one arm was just a trifle shorter than the other (see Figs. 6 and 9). In two chromosomes, however, one arm was about double as long as the other. The three remaining chromosomes presented a still larger difference in the size of the arms; in one of them one arm was a globular satellite that had the same diameter as the chromosome (Figs. 8 and 6). These observations seem to resemble rather closely those made by CHITTENDEN in root-tips of *deflexa* (see also Fig. 18). He found »four with a median

constriction, four with a submedian, six with a terminal, two with a trabant and a subterminal constriction and two with a trabant and a terminal constriction». As appears from his figures, CHITTENDEN also designates as terminal a centromere that is so far from the end of the chromosome as $^1/_3$ of the latter's length. The small trabants and secondary constrictions reproduced by CHITTENDEN could not be observed (see, however, Fig. 11), but such structures cannot be expected to show up at meiosis. Large and small trabants have also been observed in HIORTH's *deflexa* (in root mitoses). The large univalents are, further, only slightly shorter than *deflexa* chromosomes from root-tips. Their diameter, however, is several times larger, presumably owing to the fact that they have much nucleic acid at this stage. Lastly, it may be mentioned that the large univalents are of different lengths.

The region of the centromere is often plainly visible even in the small univalents. In several of them it is median, although there are also small univalents with arms of unequal length (see Fig. 6). Evidently there are differences both in shape and size also among these univalents, but they are too small to allow of close study (see also Fig. 18). The shortest *deflexa* univalent is at least $2\,^1/_2$ times as long as the largest *Bottae* univalent (in the hybrid). The small univalents are about 1,3 μ long, the large ones 3,1—4,2 μ. Strangely enough the small ones are also narrower. Their diameter is 0,5 μ while that of the large ones is 0,7 μ. Accordingly, we have here chromosomes of different diameters on the same plate. It is unexpected, but has been found only in forms in which certain chromosomes are very short so that at maximum spiralization they are shorter than the diameter of the large chromosomes.

The difference between the large and small chromosomes cannot be due to different degrees of spiralization. If the small chromosomes were shorter as a consequence of a more pronounced spiralization of the prophase threads, they ought also to be considerably thicker than the large ones. Many other things argue against the view in question. As a rule the chromosomes form an equatorial plate. When a plate contains large and small chromosomes, the latter lie centrally in it (TISCHLER, 1942, p. 183). This applies not entirely to *deflexa* × *Bottae*. As a rule a couple of small chromosomes lie at the periphery among the large ones, while a large univalent is apt to lie towards the centre of the plate (see Fig. 9). The majority of the large chromosomes, however, always lie at the periphery. An attempt was made to ascertain whether there were also other differences in the behaviour of the large

and small chromosomes. Such appeared to exist, though they were not large. Often a few univalents did not arrange themselves on the equatorial plate. This was the case with both large and small univalents, but distinctly more often with small. In this there might lie an indication that the *Bottae* chromosomes have a weaker centromere than the *deflexa* chromosomes. Another difference has reference to anaphase. The univalents did not divide at first anaphase, although there was an occasional division of a solitary univalent, in which case it was a small one. In the hybrid *Whitneyi* × *deflexa* 5 *a*, too, no univalent division was observed, while in *Whitneyi* × *deflexa* 5 *b* solitary univalents would divide once in a while (cf. HÅKANSSON, 1943). Univalents from *deflexa* 5 *a*, therefore, seem to distinguish themselves by late division of the centromere.

With reference to the first anaphase it may be mentioned that — as in haploid *G. Whitneyi* — it sometimes fails to appear because the univalents begin to undergo telophase alterations while they are still lying in the plate and therefore the latter passes direct into a restitution nucleus. As a rule, however, anaphase movement occurs. It is very irregular, some chromosomes often remaining in the plate while others are on their way to or have reached the poles. Further, there are often bridges, and these can evidently be of different kinds. Sometimes they are formed by a long univalent that has decoiled while its centromere is still in the plate. One of this kind is shown in Fig. 12; its centromere is still at the equator of the spindle, and no centromere can be discerned at the pole. This chromosome has undergone despiralization earlier than the others. Then there are bridges that appear to be formed by a bivalent whose members have not separated. Fig. 15 shows a bridge that joins two newly formed interkinesis nuclei. Probably it is an inversion bridge: there are two chromosomes in the cytoplasm, one of which, of course, may be an acentric fragment. On account of these conditions the result of anaphase may be rather different, e. g. two polar interkinesis nuclei may be formed of varying size; extra interkinesis nuclei may be formed from lagging or isolated chromosomes; a restitution nucleus of often very bizarre appearance may be formed (see Figs. 13 and 14). Relatively often a nucleus is formed with 17 chromosomes and one with one chromosome.

During telophase 1 the behaviour of the chromosomes differs from that in *Whitneyi*, the most studied *Godetia* species. In *Whitneyi* they become only slightly despiralized, and are clearly discernible during the whole stage of interkinesis. In *deflexa* × *Bottae*, on the other hand,

they are much lengthened, the chromosomes looking like long spirals during this despiralizing process, after which they become diffuse and the boundary between the different chromosomes disappears in the interkinesis nucleus, which seems to contain but very little chromatic material. It also appeared as though the interkinesis nucleus, especially the restitution nucleus, first formed many nucleoli, which later partially fused with one another. This would, in that case, represent another dissimilarity as against *Whitneyi,* in which the haploid interkinesis nucleus usually forms one or, more seldom, two nucleoli, and the diploid four. This was verified by means of special staining of some slides by BHADURI's method, kindly carried out for me by Dr. A. LEVAN. This method is not so successful when applied to *Godetia* as to many other objects, but the result is sufficiently good to ensure a decision as to whether real nucleoli are present. Fig. 14 shows a restitution nucleus together with an accessory small nucleus formed from a more remotely lying chromosome. At least thirteen nucleoli could be observed with certainty. They were of two size-classes, and a ready assumption is that the large nucleoli were formed from *deflexa* and the small from *Bottae* chromosomes. Still more nucleoli could be observed. Consequently, it almost seems as though, as in *Pisum*, a nucleolus is formed at every chromosome (HÅKANSSON and LEVAN, 1942). Strangely enough, they did not develop in *Pisum* at secondary constrictions but at or near the centromeres; in *deflexa* × *Bottae* their point of formation could not be determined.

The second division was often quite regular, though it could also often show disturbances. Fig. 16 shows how the chromosomes from a restitution nucleus orient themselves to a metaphase plate. The number is more than 18, evidently owing to the fact that a *Bottae* chromosome had divided earlier. At times an anaphase 2 was observed in a pmc in which one of the interkinesis nuclei had 17 chromosomes, the other one. It was then found that the small nucleus could be formed from a large or from a small chromosome. Bridge formation was also not uncommon at anaphase 2 (Fig. 17). Lagging chromatids were also observed.

Restitution nuclei lead to formation of pollen dyads with the unreduced number of chromosomes. The percentage of dyads evidently varied in the plants examined. In 1319 and 1320 there were not many sporads to be counted, but from meiosis it was seen that dyads were formed more often than 50 per cent. For instance, at one point in a slide 17 pmc's with restitution nucleus were counted, while in 12 pmc's

two polar groups had been formed. But the proportion varied in different parts of the slides. In 1321 the proportion of restitution nuclei was much higher, and in a couple of anthers from a bud with sporads there were 90 per cent. dyads, viz. 117 out of 128.

CONCLUSIONS.

Examination of the hybrid *G. deflexa* × *Bottae* has yielded a couple of results that are of some interest. The chief of these is that this hybrid undoubtedly has nine large and nine small chromosomes, that in respect of bulk and shape the large ones agree with the chromosomes in *G. deflexa*, and that the small ones evidently agree with the chromosomes in *G. Bottae*. The chromosomes of the parent species appear, therefore, to be practically unchanged in the hybrid. The difference in size is so considerable that a mistake is hardly possible. The observations on the different chromosome diameters are confirmed by studies of root mitoses. Retained chromosome size has also been established in another hybrid, namely *G. Whitneyi* × *Bottae* (HÅKANSSON, 1943). This could not be studied so exhaustively, but the occurrence of seven large (i. e. *Whitneyi* chromosomes) and nine small chromosomes is certain. *G. Whitneyi* and *Bottae* belong to different groups, and the hybrid shows complete asyndesis. Reference may here be made to a statement by CHITTENDEN (l. c., p. 302), which is not usually cited, probably because of its brevity. CHITTENDEN produced the hybrid between *G. deflexa* (he employs the name *Bottae*) and *tenella* (which has 16 or 17 small chromosomes). The hybrid plant obtained, which was very sterile, was pollinated with *deflexa*. The primary hybrid was not cytologically examined, but the five back-cross plants had 18 large and a number of small chromosomes in somatic plates. The primary hybrid should therefore have had nine large and 16 (17) small chromosomes, and the chromosome size to have been retained through the female gametophyte. Actually the expectation should have been a higher chromosome number in the back-cross plants than was found, since the primary hybrid had undoubtedly had complete asyndesis at meiosis, and therefore the functioning egg-cells ought to have been unreduced gametes.

A further remarkable fact is that *G. deflexa* × *Bottae* shows such strong asyndesis. In *Godetia* it is so, that hybrids between species belonging to the same species-group show relatively good chromosome pairing. When, however, a hybrid contains genomes belonging to spec-

ies from different groups, asyndesis has been found to exist in the cases hitherto examined. But *G. deflexa* and *Bottae* belong to the same group and have even been considered to have only the systematic rank of subspecies. It is however clear that HIORTH is right in regarding them as species and not subspecies. The unexpectedly strong asyndesis probably depends on the fact that homologous chromosomes have such different sizes. It is, of course, not known whether the chromosomes, in their most distended stage at prophase, can be equally long, a contingency that seems less probable, but even if this should be the case double threads formed by side-by-side pairing must presumably often fall apart into their components as a result of the unequal contraction of the two members. The bivalents that have been observed have, in fact, almost invariably only one terminal chiasma. The chromosome size would, thus, be a factor that has influence on the pairing. To this it might be objected that the *X* and *Y* chromosomes form a pair even if they are considerably dissimilar. That, however, is another kind of pairing depending on heterochromatin (*Y*, as known, can be almost empty), which cannot be directly compared with pairing between genes.

Differences in chromosome size — in cases not involving modificative alterations — are according to DARLINGTON and other investigators due to structural differences or to their being controlled by the entire genotype. Structural differences occur between the chromosomes within the same set, so that these chromosomes may have very different sizes, such differences, of course, being retained at hybridization. When, however, all the chromosomes of a species have different sizes throughout than those of a related species, this cannot be due to a structural difference. As an example of an enormous difference in size it has been mentioned that the genus *Drosophyllum* has chromosomes 1000 times as large as those of *Drosera*, a difference that must be genotypically controlled. DARLINGTON gives several examples of a genotypical control of chromosomes. »*Tradescantia brevicaulis* is a triploid, apparently a hybrid between tetraploid *Tradescantia virginiana* with large chromosomes and a diploid relative with smaller chromosomes of proportionate diameter. It has chromosomes intermediate in length and width between those of its supposed parents. In the pollen grains of one bud of the plant studied the chromosomes are proportionately reduced to one-fifth and the resting nuclei to one-third of the normal size» (1937, p. 55). The size of the cells in the bud with changed chromosomes was unchanged. Another example of alteration in chro-

mosome size through mutation is afforded by a *Lolium* hybrid. As UPCOTT (1936, p. 136) also points out in connection with a statement that *Aesculus Pavia* and *Ae. Hippocastanum* had very different chromosome sizes which were retained in their hybrid *Ae. carnea* (SKOVSTED, 1929), this difference cannot be of a structural nature. Of the species »the one cannot have uniformly lost part of each chromosome, nor can the other have uniformly gained». But if there had been a genotypically controlled difference between the chromosomes, it ought to have disappeared in the hybrid, where the chromosomes were controlled by a new genotype. UPCOTT's investigation also showed that the supposed difference in size in *Aesculus* did not exist.

In several cases the chromosomes in hybrids have been found to be under the influence of genotypical control. However, exact determinations of their volume have not been made. In some cases it has been stressed that the chromosomes of the hybrid are intermediate in both length and width to those of the parents (SIMONET, 1931, respecting an *Iris* hybrid; DARLINGTON respecting *Tradescantia brevicaulis*). In other cases the chromosomes have been found smaller in these respects in the hybrid than in the parents (see DARLINGTON, 1937, p. 55). Some investigators have emphasized that it is the diameter of the chromosomes that is intermediate in the hybrid. NAVASHIN has made detailed investigations of the size of the *Crepis* chromosomes in species and hybrids, but only part of his results seem to be published (1931). For instance, *C. capillaris* has chromosomes that are longer and of greater diameter than those of *neglecta*. In *capillaris* × *neglecta* all chromosomes have the same diameter. To attain this diametrical equality, however, the *capillaris* chromosomes have become longer, the *neglecta* shorter. LEVAN (1936) has studied *Allium cepa* and *fistulosum* and their hybrid. *A. cepa* has chromosomes that are longer and of greater diameter than those of *fistulosum*; in the hybrid, however, all chromosomes are of the same diameter. This is intermediate between those of the parents. It could not however be decided whether the *cepa* chromosomes are longer than the *fistulosum* chromosomes in the hybrid.

As evidence for the doctrine of the constancy of chromosomes many earlier textbooks and manuals cite the chromosomal relations in certain species and genus hybrids in teleostei and butterflies (see WILSON, 1925; BĚLAŘ, 1928), where as between the parents the chromosomes were of very different sizes. These differences in chromosome size were retained in the hybrid. Here, therefore, genotypic control should be absent. But the teleostean hybrids could only be studied during the cleavage

divisions of the egg, and it is quite possible that the hybrid genotype had not yet asserted its influence. The great difference between the chromosomes from *Menidia* and *Fundulus* found in the hybrid would perhaps have vanished later. The butterfly hybrids referred to developed further, and their meiosis showed large and small chromosomes. As can be seen from the figures submitted, however, both the parents had both large and small chromosomes, and it is therefore impossible to assert that the hybrid did not show genotypic control of the chromosomes.

The difference in size between the chromosomes of *Godetia deflexa* and *Whitneyi* on one hand and of *G. Bottae* on the other hand appears to be greater than that in the hybrids just cited, excepting however *Menidia* and *Fundulus*. Obviously this size difference cannot be of a structural nature, being quite too large for that, but nor is it controlled by genotypes of the respective species, for it is retained in the hybrid. A third factor seems to be active here. Thoughts turn to such accessory materials as according to DARLINGTON (1932) might account for the then unexplained *Aesculus* case. As was pointed out in another place (HÅKANSSON, 1943), the *Godetia* genus seems to possess two different kinds of chromosomes, »large chromosomes» and »small chromosomes». The extent to which these may have a different quantitative effect on cell-size, etc. requires further investigation. Measurements will have to be undertaken of different kinds of cells and of cells from different individuals. However, *G. biloba* with only $n = 8$ small chromosomes has rather large pmc's (HÅKANSSON, 1941).

The quantity of accessory material that is formed is determined by the chromosome itself. A *Bottae* chromosome develops a much smaller amount than a *deflexa* one. It would seem as though there was also a difference in the amount of the nucleolar material formed at the chromosomes. In the interkinesis nuclei of the hybrid, nucleoli were formed at many chromosomes. In some cases it was observed that these were of two size-classes. It is therefore not impossible that smaller nucleoli are formed at the *Bottae* chromosomes than at the *deflexa*, which would constitute still another quantitative difference between them.

SUMMARY.

Godetia deflexa and *G. Bottae* have been considered as subspecies or as different species. The former is a robust type with 18 large

chromosomes, the latter a slender type with 18 small chromosomes. *G. deflexa* × *Bottae* is very sterile, and has nine large and nine small chromosomes. Meiosis shows strong asyndesis, in more than 50 % of the pmc's there are nine large and nine small univalents. Their difference in size is considerable. Often, however, one or a few bivalents are formed, usually through allosyndesis but seldom through autosyndesis. The dissimilarity between the chromosomes of *deflexa* and those of *Bottae* appears to be neither of a structural nature nor genotypically controlled.

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STUDIES OF ASYNAPSIS IN RYE

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I. INTRODUCTION.

SOME years ago A. MÜNTZING and the present writer started an investigation on the cytology of population plants and inbred plants of rye, some of the results of which have already been published (MÜNTZING and PRAKKEN, 1941; PRAKKEN and MÜNTZING, 1942). Most of the inbred material studied consisted of lines which had been inbred for many years, but some I_1- and I_2-families were studied as well. The main results of the investigations on the inbred lines will soon be published and therefore the general characteristics of inbred rye will not be discussed in the present article.

In one of these I_1-families (family 100, 1940; this family as all the other material being isolated from the Swedish variety »Stålråg») an »asynaptic» plant was found, viz. a plant which showed many univalents and but few bivalents at the first metaphase in the p. m. c. In order to investigate this case of asynapsis a number of plants in family 100 and their offspring were studied, in the years 1940—1942, with respect to meiosis and pollen fertility. — Almost all fixations were made with diluted chrome-acetic formalin after prefixation with Carnoy. Staining was with gentian violet.

II. INHERITANCE.

Most though not all of the plants studied arose after self-fertilization. The results of self-fertilization in rye are, however, often very poor. In addition, extremely severe winters decreased the number of plants. In most plants both meiosis and pollen fertility were studied, in others only the one or the other. For these reasons I shall not discuss in detail the rather fragmentary material. In all, 128 plants from 20 families could be studied.

As may be seen from Table 1, the number of asynaptic plants in each of the eight segregating families (from normal mother plants) agrees rather well with a 3 : 1 segregation, suggesting a monofactorial recessive inheritance. With one possible exception, the asynaptic plants

TABLE 1. *Numbers of normal and asynaptic plants in segregating families.*

Year	No. of fam.	Normal	Asynaptic	Total	Exp. asyn.
1940	100	10	3	13	3,25
1941	292	8	1	9	2,25
1942	1	2	1	3	0,75
1942	2	1	2	3	0,75
1942	6	7	4	11	2,75
1942	7	5	1	6	1,50
1942	8	5	2	7	1,75
1942	11	1	1	2	0,50
Total		39	15	54	13,50

did not produce any seeds. This exception was a family consisting of two normal plants which were derived from a self-fertilized asynaptic mother plant. I am inclined to consider these plants as products of accidental cross-fertilization or of some mistake. Further, the proportion of eight segregating to twelve non-segregating families does not agree very well with a 2 : 1 ratio. In only five of the non-segregating families, however, the number of plants studied was four or more. Moreover, a few of them are possibly descendants from a homozygous normal mother family.

In order to trace possible differences in pairing intensity between homozygous and heterozygous normal plants, 100 first metaphase plates in each of 51 normal plants from segregating and non-segregating families were studied by counting the numbers of ring and rod bivalents. Details will not be discussed. For all plants together the results were as follows.

Mean number of rods per cell:	0	0,50	1,00	1,50	2,00	2,50	3,00	Total
Number of plants:	4	9	20	10	5	3		51

Three mother plants of segregating families belonged to the 51 plants studied. Their mean rod numbers were 1,14, 1,33 and 1,57 respectively. Heterozygosity for the asynapsis factor appeared hardly or not at all to diminish the pairing intensity.

III. MEIOSIS IN POLLEN MOTHER CELLS OF ASYNAPTIC PLANTS.

1. FIRST PROPHASE.

Leptotene and zygotene have not been studied in detail. Distinct pachytene stages were studied in both normal and asynaptic plants. The

heterochromatic terminal knobs at one end of the chromosomes (as described by LEVAN in his study on haploid rye, 1942) were often very

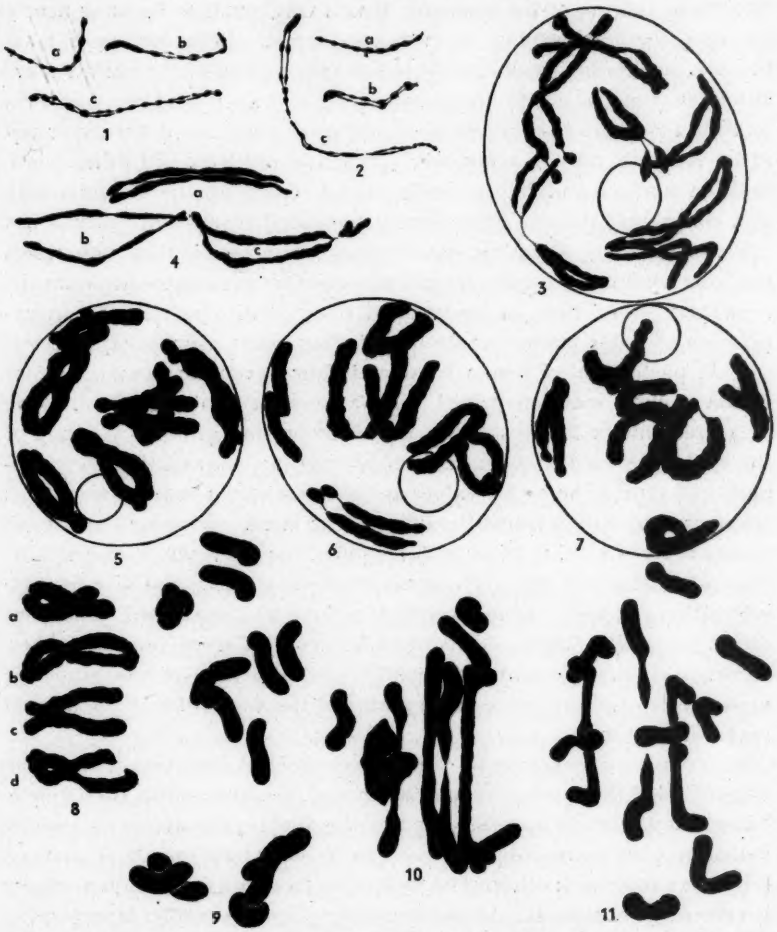

Fig. 1. Pachytene in a normal plant. — Fig. 2. Pachytene in an asynaptic plant (292—3, 1941). — Fig. 3. Late diplotene in an asynaptic plant (2—2, 1942). — Fig. 4. Late diplotene pairs, separately drawn, from the same plant as Fig. 3. — Fig. 5. Diakinesis in a normal plant. — Figs. 6—11 are from asynaptic plants. — Figs. 6 and 7. Diakinesis (292—3, 1941). — Fig. 8. Some rare types of diakinesis bivalents, separately drawn (292—3, 1941); *a* and *b* show relational coiling, *c* has one subterminal chiasma and *d* has two chiasmata. — Figs. 9—11. I—M with 0, 5 and 3 bivalents respectively (6—8, 1942, 6—8, 1942, 100—3, 1940); in Figs. 10 and 11 one of the rods has (had) the chiasma in the short arm. — × 2600.

clearly seen in normal as well as in asynaptic plants. They were, however, usually somewhat longer and narrower than in LEVAN's Fig. 2. Pachytene pairing in the asynaptic plants was found to be as or nearly as complete and regular as in normal ones. In many cases good fixation and staining made possible a detailed study of the chromomere structure (Figs. 1 and 2). In most parts, the two paired chromomeres and the two threads connecting the different pairs could hardly or not at all be distinguished separately (Figs. 1 *a* and 2 *a*). In other parts, both in normal and in asynaptic plants, however, the chromomeres and connecting threads were clearly separated from each other (Figs. 1 *b*, *c* and 2 *b*, *c*). From my observations it is probable that such places are somewhat more numerous and of somewhat greater extension in the asynaptic plants than in the normal ones. This, possibly, is an expression of a less intensive relational coiling in the asynaptic plants.

At pachytene of five normal and four asynaptic plants the size of the nucleus was determined (as the mean of two diameters). Five measurements of 20 cells each were made in both groups. A study of the figures by means of an analysis of variance showed that the probability of getting the actual values as the result of ten random samplings from one population is less than 0,001. The mean values for the normal plants were 24,90, 25,40, 25,55, 26,20 and 26,45, for the asynaptic ones 23,50, 24,30, 24,65, 24,80 and 26,65. The general means are 25,70 and 24,78 respectively ($D/m_D = 3{,}83$). I am, however, not entirely convinced that a size difference really exists. Differences between different years (weather, fixation; cf. p. 481) may have had some influence (in the asynaptic material three countings were on plants of the year 1940, in the normal material only one).

From the appearance of the chromosomes at diplotene it may also be concluded that pachytene pairing in the asynaptic plants is complete. They almost always form seven pairs, the members of each pair usually with apparent points of connection over the whole length or at least lying very near each other. The difference from normal diplotene stages is rather inconspicuous. In the asynaptic plants, however, long parallel or almost parallel chromosome parts are more often seen than in normal plants. It was impossible to count the number of chiasmata. True chiasmata could not always be distinguished from mere points of contact or from free relational coils.

After diplotene the difference between normal and asynaptic becomes more and more evident. Fig. 3 shows an asynaptic late diplotene or early diakinesis. The chiasmata are none, one or two, in most cases

terminal or nearly terminal. In some of the pairs relational coiling is easily seen. In other cases (Fig. 4) it does not occur, the chromosomes lying on the whole parallelly. The next figures show normal (Fig. 5) and »asynaptic» (Figs. 6—7) diakinesis. Normal plants almost always have seven bivalents, all clearly showing one, two or three (rarely four) chiasmata, many of which are subterminal. The asynaptic plants as well usually show seven »pairs» of chromosomes. The number of chiasmata is, however, lower than in normal plants, being none, one or two. Almost all chiasmata are terminal. In many cases the terminal connection is so weak as to raise doubts about its character as a real (terminalized) chiasma. Relational coiling has disappeared almost completely. Some infrequent types of bivalents are represented by Fig. 8. At the end of diakinesis the chromosomes become crowded in the centre of the nucleus. Even at this stage they usually form seven pairs, with or without connection between the two members.

2. FIRST METAPHASE.

Not until the nuclear membrane disappears and the spindle becomes visible does the appearance of the pollen mother cell become quite clearly asynaptic. During prometaphase chromosomes are orientated more or less parallel to the spindle. Pairs without connection may be pushed in opposite directions or they may remain close to one another. In most cases bivalents become orientated at the equator. Apparently some pairs which are connected very loosely are unable to withstand the stress exerted on them and become separated. At full metaphase (Figs. 9—12) all transitions may be found from clear bivalents over just separating and separated bivalents to chromosome pairs which lie symmetrically with respect to the equator but whose arms are not stretched and not clearly directed to each other. This last type of chromosome pairs was not counted as bivalents, and nor were the rarely occurring unorientated loosely connected pairs. These difficulties in counting the bivalents must be borne in mind in any attempt to set up statistics of bivalents and of their chiasmata at metaphase. In the greater part of the cells, however, no dubious cases occur. Rings, rods and univalents were counted in nine asynaptic plants. In almost all these plants practically every cell in the loculi studied could be analysed and involuntary selection thus avoided.

Before the results are given a few remarks must be made about the structure of the metaphase chromosomes in normal and asynaptic inbred plants. In most of the normal inbred plants the number of rods

TABLE 2. *Numbers of bivalents in nine asynaptic*

Plant number	Year	Bivalents per cell (mean)	Rings in % of bivalents	Number of cells with 0 to 7 bivalents; 0 cells	1 cells	1 % rings	2 cells	2 % rings	3 cells
6—7	1942	4,98	29,35	0	1	0,00	5	30,00	19
1—3	1942	4,40	22,50	1	5	20,00	12	8,33	29
292—3	1941	4,05	22,20	1	2	0,00	22	29,55	23
6—6	1942	3,84	21,61	1	4	0,00	11	22,72	15
11—1	1942	3,79	18,73	1	3	0,00	9	16,67	26
6—8	1942	3,72	19,07	4	10	20,00	36	26,39	74
	Total	3,96	20,78	8	24	12,5	90	23,33	167
100—5	1940	2,79	9,68	2	12	8,33	26	7,69	35
100—3	1940	2,63	12,11	25	101	10,89	171	12,28	199
100—2	1940	2,38	11,20	9	27	11,11	45	13,33	43
	Total	2,61	11,61	36	140	10,71	242	11,98	277
Grand total		3,44	18,87	44	165	10,91	337	15,28	463

is higher than in population plants, i. e. the number of chiasmata is lower. And often their chromosomes are longer and thinner, consequently less contracted. But these two characteristics, fewer chiasmata and less contraction, are not always correlated to one another (as stated also by LAMM, 1936). All asynaptic plants have chromosomes which are longer and thinner than those of the population plants. But the chromosomes in the most extreme normal inbred plants (i. e. those with the thinnest chromosomes) are thinner than those in the least extreme asynaptic plants, and thus absolute correlation is lacking here, too. The second remark concerns the number and position of chiasmata. Two chiasmata in one arm are very rare in the first metaphase of asynaptic plants. The distinction between rings, rods and univalents (with two, one and no chiasmata) therefore means but a small error in the counting of chiasmata. Most of the I—M bivalents in asynaptic plants are connected terminally and the percentages of terminal connection seem to increase with decreasing number of bivalents. In three plants, with 4,98, 3,72 and 2,74 as mean number of bivalents per cell, 703, 904 and 1041 rods were classified as having a terminal or a subterminal chiasma. The percentages with terminal chiasma were 47,80, 58,30 and 60,42 respectively.

Table 2 shows the results of all I—M countings. The nine asynaptic plants mentioned in this table are arranged according to decreasing mean number of bivalents per cell, which numbers vary from 4,98 to 2,38. The next column represents the number of rings in each plant expressed as percentages of the actual number of bivalents in the cells

plants and percentages of rings among the bivalents.

rings in % of bivalents								Total
3	4		5		6		7	number
% rings	cells	% rings	cells	% rings	cells	% rings	cells	% rings	of cells
36,84	35	25,71	69	28,41	55	31,52	16	26,79	200
21,84	56	20,09	52	23,84	35	25,24	10	22,86	200
11,59	43	21,52	37	21,62	18	25,93	4	32,14	150
15,55	37	20,27	27	25,19	5	23,33			100
19,23	34	20,59	18	15,56	9	20,37			100
22,97	91	17,86	63	17,46	20	16,67	2	7,14	300
19,96	261	19,64	197	20,81	87	22,80	16	23,21	850
12,38	17	8,82	6	10,00	2	0,00			100
11,73	110	11,82	34	15,88	10	8,33			650
10,85	19	10,53	7	8,57					150
11,67	146	11,30	47	14,04	12	6,94			900
15,69	442	17,36	313	21,47	154	24,68	32	25,00	1950

counted. These percentages lie between 29,35 and 9,68. From the table it is evident that, on the whole, with a decreasing mean number of bivalents per cell the percentage of rings among the bivalents decreases too.

In the table the nine plants are divided into three groups, with one, five and three similar plants. The mean numbers of bivalents per cell within each group are 4,98, 3,96 and 2,61, the percentages of rings 29,35, 20,78 and 11,61 respectively. The three plants in the last group, with a very weak pairing (and with very long chromosomes), are those grown and fixed in 1940. This remarkable difference between the year 1940, on the one hand, and 1941 and 1942, on the other hand, suggests the influence of weather conditions rather than of genetical differences. As the material was grown at Svalöf, detailed meteorological observations were available. From a study of the records it became evident that all fixations in 1940 were made at a high temperature under a long period of drought. None of the fixations in the next years were carried out under such extreme conditions. A close comparison of the meteorological records with the metaphase pairing intensities suggested that especially the relative humidity of the soil and of the air influences the pairing.

In the same Table 2 a list is made for each plant of the numbers of cells with none to seven bivalents respectively, and for each category of cells the percentage of rings among the bivalents in these cells is given. Within each plant the numbers of cells with none to seven bi-

valents show a fairly normal frequency distribution. In the plant with the highest mean number of bivalents per cell (6—7, 1942) no cells with only univalents were found, whereas in the three plants with low mean number (100—2, —3 and —5, 1940) no cells with only bivalents occurred. The percentages of rings in the categories with one to seven bivalents show, within each plant, a rather high variability. However, a clear tendency to increase the percentage of rings simultaneously with increasing number of bivalents per cell (as might be expected if in each individual cell the chiasmata present were distributed at random over the fourteen arms) does not occur. Adding the plants into the three groups mentioned above (with 200, 850 and 900 analysed cells) it becomes clear that, on the whole, the percentage of rings, within each group of plants, is practically the same in all of the cell categories with one to seven bivalents and, therefore, about the same as the general percentage within each group, viz. 29,35, 20,78 and 11,61 per cent (cf. Table 2). The lowest line in the table represents the values for all plants together. Here, owing to the inter-plant correlation between mean number of bivalents and percentage of rings, the cell categories, naturally, show with increasing number of bivalents also an increasing percentage of rings, from 10,91 to 25,00 per cent.

The fact that, within each plant (or group of similar plants), the percentage of rings is about the same in all the cell categories with one to seven bivalents means that the individual cells contain something like random samples from the total bivalent population. This is clearly borne out by an analysis of variance of chiasma frequencies. The calculations have been made for three groups of 200 cells, representing one plant from each group, with the following result.

Plant	Mean square within cells	Mean square between cells	v^2 obtained	v^2 P = 0,2	v^2 P = 0,05	v^2 P = 0,01
6—7, 1942	0,488	0,513	1,05			
1—3, 1942	0,444	0,558	1,26	1,14	1,19	1,27
100—3, 1940 [1] ...	0,293	0,295	1,01			

In all three cases the mean square »between cells» is but slightly and not significantly higher than »within cells», the P-values being $> 0{,}2$, $0{,}05 > P > 0{,}01$ and $> 0{,}2$ respectively. In the remaining six plants the situation is about the same.

[1] From a fixation of plant 100—3 with a lower number of bivalents than the fixation represented in Table 2.

Operating, finally, with all the counted chiasmata from a plant, the numbers of chromosome pairs with none, one and two chiasmata appear to correspond rather accurately with a random distribution over the fourteen arms. This is shown by the following three examples, which refer to the same three groups of 200 cells as mentioned above.

Plant	Number of cells	Number of chiasmata		Univalent pairs	Rods	Rings
6—7, 1942	200	1287	expected.	408,24	695,52	296,24
			observed	405	703	292
1—3, 1942	200	1078	expected	529,52	662,97	207,52
			observed	520	682	198
100—3, 1940 [1]	200	517	expected	930,72	421,54	47,74
			observed	925	433	42

All statements concerning random distribution of chiasmata and its meaning, however, must be taken cautiously. Not all chromosomes in rye have the same size, and three pairs of them have a clearly subterminal centromere and thus arms of very different length. Just with respect to these chromosomes with a long and a short arm a further interesting observation was made. When some rods occur in cells of normal plants, a short arm often lacks a chiasma. Certainly, it may also be lacking in one arm of the chromosomes having a median centromere, but very rarely do the long arms lack chiasmata. An extreme case is illustrated in Fig. 14, representing a cell from an F_1 plant between two inbred lines. In this cell the three rods are the three pairs with a submedian centromere (individually identifiable in this special case; cf. PRAKKEN and MÜNTZING, 1942, Figs. 120—121 and pp. 470—471); in all three rods it is the short arm which lacks a chiasma. Figs. 11—13, on the other hand, were taken from a plant with high asynapsis (100—3, 1942). The two lateral rods in Fig. 11 are of the normal type. The third one, however, shows a peculiar rod type which has (had) the chiasma in the short arm. Fig. 13 represents single bivalents of the same type. Fig. 12, finally, is a very extreme and rare case. Among the four (separating) rod bivalents all three pairs with a clearly submedian centromere are present and all three have (had) the chiasma in the short arm. In normal plants this bivalent type is rarely seen, whereas in highly asynaptic plants it rather frequently occurs. Fig. 10 was taken from a less extremely asynaptic plant. The meaning of this structure will be dealt with in the discussion.

[1] See foot-note on p. 482.

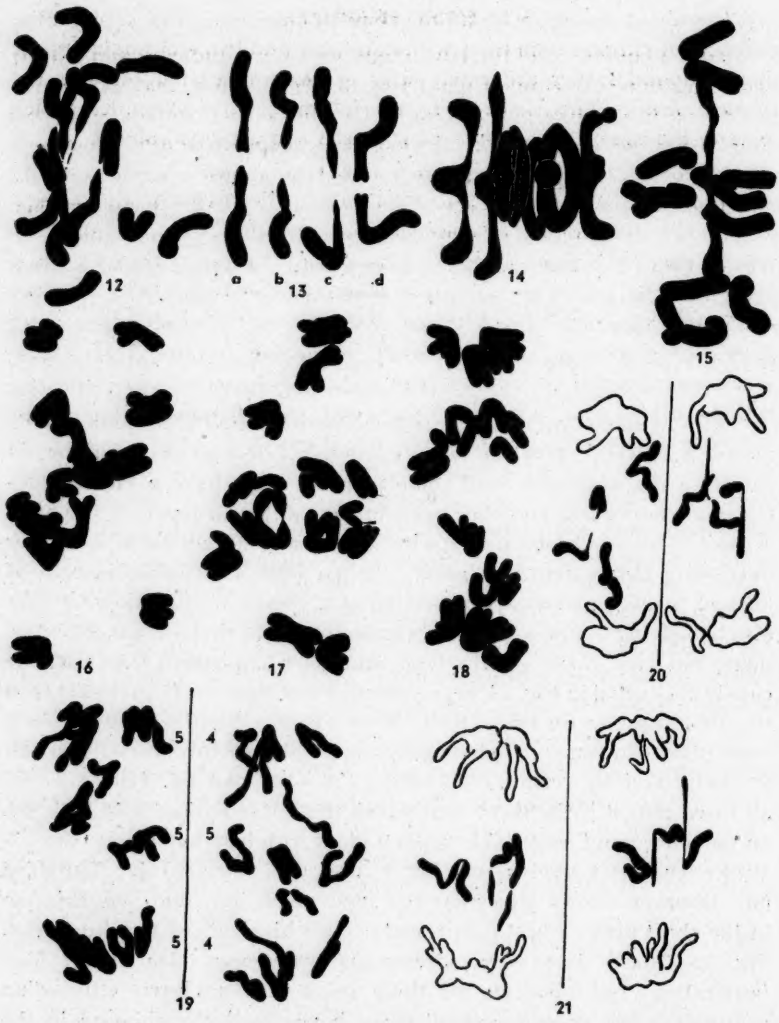

Fig. 12. I—M in an asynaptic plant (100—3, 1940); in three of the four bivalents the chiasma is (was) in the short arm. — Fig. 13. Bivalents which had the chiasma in the short arm, separately drawn (100—3, 1940, as Fig. 12). — Fig. 14. I—M in a normal plant; in the three rods chiasmata are lacking in the short arms. — Figs. 15—21 are all from the asynaptic plant 100—3, 1940. — Fig. 15. Late I—M with 1_{II} and with half of the univalents orientated at the equator at right angles to the spindle. — Fig. 16. Early I—A with two former bivalents and probably six dividing univalents. —

3. FIRST ANAPHASE.

First anaphase (and the later stages too) were studied mainly in the above-mentioned fixation of plant 100—3, 1940, with a very high degree of asynapsis (cf. foot-note p. 482). On the whole, the course of the anaphase seems to be strongly controlled by a spindle that is practically always normal. At late metaphase and early anaphase many univalents, when not lying too close to one of the poles, move towards the equator. At the same time most univalents orientate themselves at right angles to the spindle. These stages are represented by Figs. 15 and 16. The univalents that reach the equator first usually divide first also. In many cases the appearance of such first anaphases with dividing univalents between the polar groups (consisting of members of former bivalents and some univalents) is rather regular (Figs. 17 and 18). Ninety I—A cells were completely analysed. The numbers of dividing (in some cases probably dividing) univalents were as follows:

Number of dividing univalents:	0	1	2	3	4	5	6	7	8	9	10	Total
Number of cells:	1	2	10	7	16	16	18	10	5	3	2	90

The mean number of dividing univalents per cell is 5,02. In the same fixation the mean number of bivalents was 2,38, resulting in 9,24 univalents per cell. Thus, on an average, more than half of the univalents divide. At telophase lagging chromosomes occur but rarely. Bridges and fragments were very seldom seen.

4. INTERPHASE.

The appearance of the interphase is rather normal, as might be expected from the course of the first division. A unicellular interphase with a restitution nucleus was never seen and more than two cells very seldom occur. The two nuclei often show a different size. Micronuclei are found in some of the cells. One hundred interphases from plant 100—3, 1940, were of the following constitution.

Two cells			Three cells	Four cells	Total number interphases
0 micronuclei	1 micronucleus	2 micronuclei			
69	26	2	2	1	100

Figs. 17—18, I—A with seven (to nine) and six dividing univalents respectively. — Figs. 19—21, II—A. Fig. 19 with five lagging chromosomes (half univalents) at each side, Figs. 20 and 21 with three. In Figs. 20—21 the ends of some arms seem to be attracted by the pole. — × 2600 (Fig. 14, × 3100).

5. SECOND DIVISION.

The same extremely asynaptic fixation of plant 100—3, 1940, was also used for the study of the second division. In consequence of the rather numerous dividing univalents at first anaphase the second anaphase shows lagging chromosomes in almost all cells. A typical case is illustrated in Fig. 19. The constitution of its cells is 5—5—5 and 4—5—4. In the 50 cells of 25 analysed p. m. c. the mean number of lagging univalents per cell was 2,96 (the number of dividing univalents at I—M in the same fixation was 5,02). Though many of the 25 cell-pairs were analysed completely, no clear cases of univalents splitting longitudinally in both divisions were found. Another abnormal behaviour of the lagging chromosomes was, however, observed. In many cases one arm was directed towards the pole and much stretched (Figs. 20 and 21). At telophase the stretched arm sometimes forms a bridge. The phenomenon resembles the phenomena described earlier by PRAKKEN and MÜNTZING (1942). In a few cases transverse division of lagging chromosomes was observed (Fig. 20, to the left).

The »tetrads» are very irregular. In most of them the four main nuclei and cells are recognizable. But almost all 25 tetrads studied in one loculus possessed extra nuclei, in most cases micronuclei. The numbers of extra nuclei (or possibly extra cells) in these 25 tetrads were:

Number of extra nuclei per tetrad:	0	1	2	3	4	5	6	Total
Number of tetrads:	1	1	6	6	5	4	2	25

The mature pollen in asynaptic plants always has a very irregular appearance. The pollen fertility (judged after staining with acetic-carmine; classification often difficult) was:

Percentage »good» pollen	0—10	10—20	20—30	30—40	40—50	50—60	60—70	70—80	80—90	90—100	Total
Population plants (from the original population)									1	6	7
Normal inbred plants					4	4	10	27	33		78
Asynaptic inbred plants	1	—	4	2	2	2					11

IV. MEIOSIS IN EMBRYO-SAC MOTHER CELLS OF ASYNAPTIC PLANTS.

Diakinesis, first metaphase and first anaphase could be studied in one cell each. They showed the same characteristics as the pollen mother cells. In some of the ovules the embryo-sac develops rather normally, in others it degenerates.

V. DISCUSSION.

Most cases of asynapsis (i. e. decreased chromosome pairing; usually observed as the occurrence of a variable number of univalents at first metaphase) belong to one of the following groups.

1. Asynapsis due to the action of a distinct gene or genes (or some slight structural change).
2. Asynapsis caused by loss of a chromosome pair.
3. Asynapsis induced by external conditions.
4. Asynapsis as a normal process in apomictic organisms.
5. Asynapsis depending upon mechanical chromosome conditions (structure, number).
6. Asynapsis in species hybrids.

In the present discussion only the first group (to which the rye case belongs) will be dealt with in any detail, the second to fourth groups will be merely touched upon, and the last two groups will be left out of consideration.

The cases of (probably) genically caused asynapsis in plants show widely different characters. Tentatively they may be arranged into an »intensity series» by means of the numbers of univalents (rods, rings, chiasmata) at first metaphase. Cases in which monofactorial recessive segregation of the asynaptic type has been proved or made probable are indicated by *m.*

1. Weak asynapsis: a few univalents in some of the cells.
   m. Matthiola incana (MANN LESLEY and FROST, 1927; ARMSTRONG and HUSKINS, 1934).
   Secale cereale (LAMM, 1936; in inbred rye).
   m. Hordeum vulgare (EKSTRAND, 1932).
   Alopecurus pratensis (JOHNSSON, 1941).
   Sorghum sp. (HUSKINS and SMITH, 1934).
   m. Nicotiana silvestris (GOODSPEED and AVERY, 1939; extremely variable).

2. Medium strong asynapsis: many univalents in most of the cells.
   Crepis capillaris (HOLLINGSHEAD, 1930; RICHARDSON, 1935).
   m. Secale cereale (PRAKKEN, the present article).
   Pisum sativum (KOLLER, 1938).
   m. Nicotiana tabacum (R. E. CLAUSEN, 1931).
   m. Zea mays (BEADLE, 1930 and 1933).
   Rumex acetosa (YAMAMOTO, 1934).

3. Complete asynapsis: with univalents only or some rare bivalents.
   m. Datura stramonium (BERGNER, CARTLEDGE and BLAKESLEE, 1934; BLAKESLEE and AVERY, 1934).
   Hevea brasiliensis (RAMAER, 1935).
   m. Alopecurus myosuroides (JOHNSSON, 1941).
   Allium amplectens (triploid; LEVAN, 1940).

In the first category, with weak asynapsis and, thus, with few cytological irregularities, sterility is usually not high. In the second and third category, on the contrary, sterility is very high or complete (excepting perhaps *Allium*; cf. below). To high asynapsis a series of irregularities are usually correlated throughout all stages, beginning with first metaphase, viz.: few or no bivalents at I—M — a defective spindle with various abnormalities (long, bent, tripolar or double) — no movement of univalents to the equator — rare splitting of univalents at I—A — interphase often with other numbers than two nuclei or cells (one, three, four) — regular second division (save for deviating cell and chromosome numbers) — high sterility. All these characters are, on the whole, most clearly found in the group with complete asynapsis: *Datura*, *Hevea*, *Alopecurus*; also in *Zea*. In most of the remaining species with a medium degree of asynapsis these characters are less pronounced and the course of the divisions is as follows (*Rumex*, *Pisum*, *Crepis* and, most typically, *Secale*): a variable number of bivalents at I—M — a normal, effective spindle — moving of some of the univalents towards the equator — splitting of these univalents at I—A — interphase almost always with two nuclei or cells — irregular second division with lagging chromosomes — high sterility. *Allium* does not at all fit in the system and shows peculiar characters quite unparallelled in the remaining asynaptic plants: practically no bivalents but a quite normal spindle and usually an orientation of all univalents into the equator — no division of the centromeres, however, and no anaphase movement at all — unicellular interphase — quite regular second division giving dyads. (So far very little is known about the propagation in this asynaptic triploid *Allium amplectens*. It might be considered to belong to the apomictic group, but LEVAN (unpubl. data) in crossing it with a tetraploid form, ♀ $2n = 21 \times ♂ 2n = 28$, obtained a plant with $2n = 35$).

The classification according to the numbers of univalents and bivalents at I—M is, however, rather arbitrary, for in many cases the metaphase pairing is highly influenced by external conditions, modifying genes, or chromosome abnormalities. An extreme example of this is formed by *Nicotiana silvestris*. In the present case of asynaptic *Secale* espec-

ially insufficient water supply seems to increase the degree of asynapsis. Insufficient water supply is also very effective in causing decreased metaphase pairing in normal plants (OEHLKERS, 1937, KISCH, 1937).

One may suppose that, in some cases at least, the decreased metaphase pairing depends upon physical changes in the chromosomes, for instance, a higher viscosity, interfering with the chiasma formation between the paired chromosomes at pachytene-diplotene (WHITE, 1934), but not necessarily decreasing the zygotene-pachytene pairing. In many of the plants with medium or complete asynapsis at metaphase a normal prophase pairing actually occurs. Complete pachytene pairing was observed in *Crepis*, *Allium* and *Secale* (the present paper) and it was probable in *Pisum*. *Nicotiana silvestris* (with a highly variable degree of asynapsis) also showed a quite normal pachytene. In all these cases of complete pachytene pairing few or no chiasmata are formed and during diplotene-diakinesis the chromosomes gradually fall apart, usually showing, however, relative coiling in the younger stages and an orientation into pairs of univalents up to the end of diakinesis. In *Zea*, too, zygotene pairing seems to be complete (normal »synapsis» during the »synizesis» stage), but, according to BEADLE, the falling apart already occurs at pachytene and may be rather complete at early stages (1933, Fig. 22). Nevertheless, his diakinesis figures (1930, Figs. 2 and 29; same cell) clearly show many pairs of univalents.

Decreased metaphase pairing depending on a reduction in the number of chiasmata is often ascribed to disturbance of the normal time-relation between the development of chromosomes and cell. According to the precocity-theory of reduction division (DARLINGTON, 1937), a first meiotic prophase beginning too late gives the chromosomes time to divide longitudinally and therefore they are unable to pair. According to the retardation-theory of reduction division (SAX and SAX, 1935), on the contrary, a first meiotic prophase beginning too early (or proceeding too fast) interferes with the despiralization of the chromosomes and so prevents the pairing. In the above-mentioned cases the zygotene-pachytene pairing is not prevented. The possibility remains, however, that some disturbance in time-relation shortens the duration of the pairing period and in this way diminishes the number of chiasmata. In asynaptic rye plants relative coiling during pachytene-diplotene is probably somewhat less intensive than in normal plants. In many apomictic plants with totally asynaptic p. m. c. (at I—M) it has been shown that the first meiotic division begins at an abnormally early stage (cf. GUSTAFSSON, 1942). In rye pachytene nuclei were

measured but no conspicuous differences were found between normal and asynaptic plants.

In a few of the cases with medium or complete asynapsis at I—M lack of prophase chromosome pairing is, in fact, reported. According to YAMAMOTO, zygotene pairing is not found in his asynaptic *Rumex* plant. RAMAER, in an asynaptic *Hevea* clone, finds that the contents of the nucleus after synizesis consists of thin, unpaired threads; though homologous chromosomes lie side by side in various places no pairing takes place. One of the cases of asynapsis caused by loss of a chromosome pair will be mentioned here [1]. In asynaptic fatuoid *Avena* dwarfs with 40 chromosomes HUSKINS and HEARNE describe the zygotene-pachytene stages as having incomplete pairing and showing a very irregular contraction. The lack of pairing is correlated to premature splitting of the chromosome threads. Here I may draw attention to the fact that in some of the figures representing diakinesis in *Rumex* and *Avena* (and to a less degree in *Hevea*) many clear pairs of univalents can be seen. This suggests that the fundamental difference between the asynaptic forms with and without regular zygotene pairing is less profound than might be concluded from the zygotene-pachytene descriptions. In both cases disturbed time-relations may play a rôle.

In this connection attention should be drawn to the remarkable change in pairing frequency between the long and the short arms of the three chromosome pairs with a clearly submedian centromere in rye. In normal plants chiasmata are rather often lacking in the short arms, but seldom in the long ones. In highly asynaptic plants, on the contrary, the few chiasmata present are rather often found in the short arms instead of in the long ones (cf. Figs. 10—13). This »reversed» chiasma frequency may be explained in such a way that a definite part of the short arm strongly maintains its ability of forming chiasmata, whereas this ability decreases considerably in the remaining parts of the chromosome. This decrease, again, may be supposed to be caused by a »change in viscosity» of the chromosomes or by »disturbed time-relations», as discussed above.

What part of the chromosome retains its original ability? The probable answer is to be found in LEVAN's investigation on haploid rye

[1] Asynapsis due to loss of a chromosome pair has been reported in the following species: *Primula kewensis* (NEWTON and PELLEW, 1929), *Viola Orphanidis* (possibly; in the p. m. c. only; J. CLAUSEN, 1930), *Triticum* and *Avena* (cf. HUSKINS and HEARNE, 1933), and in *Godetia Whitneyi* (as the first case of a nullosomic in diploid plants; HÅKANSSON, 1943).

(1942). By means of cold treatment he demonstrated that in one arm of at least five of the seven I—M chromosome pairs small terminal heterochromatic regions occur, corresponding to the terminal knobs at prophase. In the chromosomes with a clearly submedian centromere this region is always the tip of the short arm. Thus, the chromosome parts which maintain their chiasma-forming ability in all probability correspond to the terminal heterochromatic regions or to euchromatic regions lying close by. In this way some peculiarities in haploid rye also will find their explanation (LEVAN, 1942, p. 187), and once more the importance of heterochromatin for chromosome pairing is demonstrated (cf. KOSTOFF, 1938; RIBBANDS, 1941; BOLLE and STRAUB, 1941). When studying the rod bivalents in LEVAN's Fig. 4 (about 20 in number) it is easily seen that in at least seven of them the connecting chiasma lies in the short arm, in almost all cases terminally. In the present connection the fact must be remembered that with decreasing number of bivalents (chiasmata) in asynaptic rye plants the percentage of terminal chiasmata increases (p. 480). For inbred versus population rye the same relation was found by LAMM (1936). From Figs. 10—13 in the present paper it is clear that rods which are connected by a chiasma in the short arms already begin to separate during metaphase. It is even probable that some of the terminal diakinesis connections (always true terminalized chiasmata?) are already disrupted during prometaphase (cf. also LAMM, 1936).

The last question to be discussed concerns the relative numbers of univalents, rods and rings in the asynaptic plants and cells, i. e. the distribution of the chiasmata (the rare cases of two chiasmata in one arm not being taken into account). In three plants the actual figures for all univalent pairs, rods and rings in 200 analysed cells together were in rather good agreement with a random distribution of the chiasmata over the fourteen arms, and also in the remaining plants the situation is apparently the same (cf. p. 483). Nevertheless, it must be realized that a random distribution over the arms will tend to be disturbed by various factors, some of which are working in opposite directions: different chromosome size, different length of the two arms, arms with and without heterochromatin. In agreement with a random distribution is, of course, the fact that with an increasing mean number of bivalents per cell in different plants the percentage of rings among the bivalents becomes higher (cf. the first columns in Table 2). In asynaptic maize the same correlation exists (BEADLE, 1933, Table 2). When taking together all cells from all plants this inter-plant correlation

will naturally be expressed as a positive correlation between number of bivalents per cell and mean number of chiasmata per bivalent in each of the cell categories with one to seven bivalents. For rye this correlation is clearly seen as an increasing percentage of rings among the bivalent groups at the bottom line of Table 2, and for maize it is shown by text-fig. 3 in BEADLE, 1933.

In five asynaptic *Pisum* plants KOLLER (1938) studied the same relations and he writes: »The data suggest that in asynaptic *Pisum* there is an increase of chiasma frequency per bivalent with the increase of bivalents per pollen mother cell. A similar correlation was found in maize». In the case of maize, however, the correlation shown by BEADLE's text-fig. 3 undoubtedly represents in the first place an inter-plant correlation. KOLLER, on the contrary, does not find an inter-plant correlation (his five plants having about the same bivalent and chiasma frequencies) but an intra-plant correlation. The graphs for three of his five plants (text-fig. 67) suggest an increase of chiasmata per bivalent from 1 to about 1,5. A calculation based on the data in his Table VII, however, shows merely an increase from about 1,2 to about 1,4 for 254 cells of the five plants taken together. In the present case of rye there is no intra-plant correlation at all between number of bivalents per cell and mean number of chiasmata per bivalent: within each plant or group of similar plants the percentage of rings is about the same in all of the cell categories with one to seven bivalents. This means of course that in the individual cells the distribution of chiasmata over the arms does not correspond with a random distribution: in cells with few bivalents the percentage of rings is too high, in cells with many bivalents it is too low.

Similar questions concerning chiasma numbers per chromosome and per cell (in normal, not asynaptic plants) are discussed by some other workers. Within population plants of rye, MATHER and LAMM (1935) report a »negative correlation of chiasma frequencies», i. e. they find the intra-cell variance in number of chiasmata greater than the inter-cell variance. This negative correlation is explained by MATHER (1936 and 1939) as due to a competition between bivalents during chiasma formation. A »positive correlation of chiasma frequencies», i. e. an intra-cell variance which is smaller than the inter-cell variance, is considered to depend upon »environmental influences» such as, for instance, the position of the nucleus in the anther. In inbred rye (LAMM, 1936), which has a lower chiasma frequency than population plants, no clear negative correlation was found, a few plants even

showed a significant positive one. The chiasma numbers in asynaptic *Pisum* also suggest the occurrence of a similar positive correlation, the significance of which, however, cannot be judged. For three plants in the present asynaptic rye material (with low, medium and high asynapsis) calculations of variance were made (cf. p. 482). In all three cases the inter-cell variance is slightly greater than the intra-cell variance, but in no case is the deviation significant. The chromosome pairs in the individual cells of asynaptic rye plants thus seem to represent random samples from all the chromosome pairs in the plant, and neither »competition» between chromosomes nor »environmental influences» (for the individual cells) seems to be of importance.

VI. SUMMARY.

1. A case of monofactorial recessive asynapsis in rye was studied in three generations.

2. In the pollen mother cells zygotene-pachytene pairing is complete.

3. A decreased number of chiasmata is formed and during diplotene-diakinesis many pairs fall apart to univalent pairs.

4. In I—M rod bivalents of highly asynaptic plants probably most of the connections (chiasmata) are between arms with a heterochromatic end region.

5. The mean number of bivalents per cell at I—M in nine plants ranged from 2,38 to 4,98, and almost parallelly the percentage of rings among the bivalents ranged from 9,68 to 29,35.

6. These differences between the asynaptic plants were at least partly caused by external conditions, in the first place humidity of soil and air.

7. Within each plant (or group of similar plants) the mean percentage of rings among the bivalents is about the same in each of the cell categories with one to seven bivalents. Inter-cell variance in number of chiasmata is not significantly higher than intra-cell variance.

8. A quite normally developed spindle controls the first meiotic division. In one plant with high asynapsis more than half of the univalents divided longitudinally.

9. The interphase usually shows two cells with one nucleus each, micronuclei being rather rare.

10. In the very irregular second division numerous lagging chro-

mosomes occur and the »tetrads» contain many extra micronuclei or microcytes.

11. Pollen fertility in the asynaptic plants lies between 1 and 59 per cent, in their normal sister plants between 55 and 99 per cent.

12. Embryo-sac mother cells show the same asynapsis as the pollen mother cells.

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ABSTRACTS — KURZE MITTEILUNGEN

GUNNAR ÖSTERGREN and ALBERT LEVAN: The connection between c-mitotic activity and water solubility in some monocyclic compounds.

The regular negative correlation found by us (LEVAN and ÖSTERGREN, 1943) between c-mitotic activity and water solubility in the naphthalene series prompted us to investigate whether the same regularity also prevailed in other instances. As SIMONET and GUINOCHET (e. g. 1939) had observed weak c-mitotic effects (called paradichlorobenzene effect) induced by some benzene derivatives, it was close at hand to choose a series of such substances for our purpose. Two cyclohexane derivatives were included in the experiments in order to test whether a hydrogenation of the benzene nucleus would inhibit the activity. Three thiophene compounds were also tried, thiophene being closely similar to benzene in many properties, and only very few heterocyclic substances having been studied previously. As BAUCH (1941) had produced giant races of yeast (considered as polyploids) by treatment with camphor, it was considered of some interest to study camphor and borneol, too. The methods used were the same as those employed in our previous study, and the material in this case, too, was *Allium Cepa*.

To give additional support to the considerations presented by us in our first paper on this subject (l. c.) we consider it expedient to submit, thus early, a brief account of our results from the present experiments.

The benzene series. — The substances tested and some of the results obtained may be seen from the following table. The numbers represent concentrations in 10^{-6} mol, + means full c-mitosis, ± partial disturbances, and — normal mitosis.

	Solubility		C-mitosis		
	Precipitate	Clear solution	+	±	—
Benzene	?	10000	?	3000	1000
Monochlorobenzene	1000	300	1000	300	100
Monobromobenzene	1000	300	1000	300	100
Monoiodobenzene	1000	300	1000	300	100
Parachlorotoluene	1000	300	1000	300	100
Metadichlorobenzene ...	1000	300	300		100
Metabromotoluene	1000	300	300		100
Orthodichlorobenzene ..	1000	300	300	100	30
Orthochlorotoluene	1000	300	300	100	30
Metachlorotoluene	1000	300	300	100	30
Trichlorobenzene (1, 2, 3)	100	30	100		30
Hexachlorobenzene	50	10	50	10	3

We have not, as yet, studied a saturated solution of benzene. It seems very probable that benzene, too, is able to induce full c-mitosis, as this was observed in the case of all the other benzene derivatives studied, at least in their highest concentrations.

The negative correlation between solubility and activity clearly holds here, too. In the benzene derivatives *the independence in occurrence of the*

c-tumour and the c-mitosis is even more evident than in the naphthalene series. In many cases it was found that solutions which were too weak to give any mitotic disturbances were still able to induce pronounced c-tumours. The strongest solutions were regularly unable to induce c-tumours owing to the strong, growth-inhibiting poison effect.

The cyclohexane series. — SHMUCK and GUSSEVA (1941) claim that the c-mitotic substances lose their activity when the aromatic nucleus is hydrogenated. With the favourable methods and material used by us we have, however, been able to demonstrate a c-mitotic activity also in cyclohexane and bromocyclohexane. *It was even found that cyclohexane is active in a much lower concentration than benzene*, an observation which is in accordance with the difference in water solubility between these two substances. This fact also disproves any suggestion that the activity of cyclohexane should be due to an impurification of the tested substance by benzene. Cyclohexane, however, was only able to induce a partial c-mitosis, even in saturated solutions. The difference between cyclohexane and the benzene derivatives, responsible for the absence of complete c-mitosis, probably consists in a different size of the active concentration range, this being much smaller in cyclohexane. Consequently, the difference may be said to be of the same kind as between the α- and β-derivatives of naphthalene. Bromocyclohexane, however, was able to induce full c-mitosis.

The solubility of cyclohexane is between 1000 and 500×10^{-6} mol, and its threshold of action between 500 and 300. The corresponding values for bromocyclohexane are 500—300 and 300—100. Thus, the solubility—activity correlation is also valid here.

The thiophene series. — As thiophene is closely similar to benzene, it was not surprising to find that this substance also has c-mitotic properties. The results from the thiophene series are as follows (conc. 10^{-6} mol):

	Solubility		C-mitosis		
	Precipitate	Clear solution	+	±	−
Thiophene	5000	2000	5000	2000	1000
Dibromothiophene ..	100	50	300	100	50
Diiodothiophene	30	10	100	30	10

Thus, the results indicate the same kind of regularity as was found in our other series.

Camphor and borneol. — These two terpene derivatives were both found to induce full c-mitosis in their stronger concentrations, and the correlation studied was found to be valid here, too. The solubility of camphor is between 10000 and 5000×10^{-6} mol and its threshold of action between 1000 and 650. The corresponding numbers for borneol are 4000—2000 and 400—200.

Conclusion. — The results strongly support the view presented by LEVAN and ÖSTERGREN (l. c.) that the c-mitotic properties of organic substances are not due to their chemical properties (their reactivity, etc.), but to their *physical properties.* If the water solubility is not, itself, the decisive property (which seems but little probable), it can at least be stated that the decisive property must be one that is correlated in some way or other to the solubility.

In our previous paper some detailed suggestions were submitted as to the mechanism. We are well aware that these considerations are of rather a

preliminary nature. Anyhow, the empirical connection discovered by us may be considered as a beginning to a deeper understanding of the mechanism of chemical disturbances of cell division.

Lund and Svalöf, April 1943.

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O. HEILBORN: Inversions in *Paris quadrifolia*.

In two recent papers (Zeitschr. f. ind. Abst.- und Vererbgsl., 73, 1937, and 75, 1938), GEITLER has analyzed numerous cases of inversion heterozygotes in *Paris quadrifolia* from natural populations at Lunz, in Austria. The inversions become visible as chromosome bridges and fragments, caused by crossing-over in the inverted regions. At least 45 different types of such inversions were recognized. All chromosome arms in the nucleus showed inversions, as demonstrated in some forty plants. The frequency of inversions in the various plants was different, varying from 1,2 to 12,3 % of the pollen mother-cells investigated, mostly 2—6 % of the pollen mother-cells showing inversion pictures. Thus, generally, the frequency was rather high. — Univalent chromosomes, too, occurred regularly. Small percentages (1,2—1,7 %) of pollen mother-cells with univalents were generally found, in some of the plants these percentages were high (4—16,7 %), and in one single plant it was very high (73,5 %).

The regular occurrence and high frequency of inversions in all chromosome arms of the Austrian material led GEITLER to the suggestion that these inversions might be very old, possibly dating from a time before the species had yet become tetraploid. If this be so, the investigation of material from various countries and stations within the distribution area of the species would seem desirable. If the inversions are old, they should probably occur over wide areas, their occurrence at Lunz not being a local one. In order to test this problem, the present writer last spring collected material of *Paris quadrifolia* at Djursholm (park of Svalnäs) near Stockholm. The station is an alder grove at the sea-shore. The population is probably an old one, as no distinct limits between the various colonies, propagated vegetatively, could be discerned. Consequently, it could not be decided whether the flower buds collected were derived from distinct clones or not. Therefore, in the following no attempt is made to separate the various plants; only the numbers of the slides are noted.

Eleven slides, containing anaphases and telophases in first division of the pollen mother-cells have been investigated. Of these, six show inversion pictures, in five such figures could not be found. In the slides, showing inversion pictures, their frequency is very low — always less than 1 % of the pollen mother-cells investigated. However, though the frequency is low, the regular occurrence of inversions in this material is quite obvious. Univalents occur in all the slides and are distinctly more numerous than the bridges. The occurrence of inversion bridges in the six slides is as follows:

Slides:	I	II	V	VI	VII	X
Number of bridges:	3	2	3	2	2	3

Inversion pictures thus occur in this Swedish material as well as in GEITLER's Austrian one though by no means so frequently. The lower frequency of inversion pictures may depend upon: (1) a lower frequency of inverted sections; (2) less heterozygous conditions of the plants; or (3) a lower frequency of chiasma-formation and crossing-over. The regular occurrence of univalent chromosomes speaks in favour of the last-mentioned explanation. Univalents always indicate a weak chiasma-formation and, consequently, a reduced crossing-over. The second explanation would seem less likely, considering the strong vegetative propagation of the plants which would promote heterozygosity. It is more difficult to estimate the probability of the first explanation.

The occurrence of inversions in widely separated parts of the distribution area of *Paris quadrifolia* may well be interpreted as indicating a high age of the inversions, but this interpretation is not necessarily the correct one. The cause may also be sought in some special liability to produce inversions, new inversions then appearing repeatedly at various localities during the evolution of the species.

TYGE W. BÖCHER: Polyploidy in the genus *Koeleria*.

The present investigation was made in order to study the Danish species of *Koeleria* from a cytogenetic point of view. In his paper on the Danish *Koeleriae* DOMIN (1906) describes two wild-growing species, viz. *K. glauca* DC. var. *intermedia* (AHLQ.) DOMIN and *K. pyramidata* DOMIN var. *danica* DOMIN. Both species are represented in Denmark by special varieties, but the var. *intermedia* also occurs in Sweden and in some localities along the Baltic Sea. The Danish population of *K. glauca* var. *intermedia* is isolated from more eastern populations by a gap extending from West Jutland to Scania.

According to DOMIN (1907) *K. glauca* and *K. pyramidata* must be regarded as collective species, the variation in both species being very wide. DOMIN states that cultivation experiments are of very limited value for taxonomic investigations in the genus. The present writer, however, thinks that satisfactory results can only be obtained by means of combined cytogenetic and taxonomic studies.

In the case of *K. glauca* three different types have been found. The Danish dune race (var. *intermedia*) is diploid ($2n = 14$) and cytologically it is almost identical with var. *typica* (Fig. I, p. 201 in AVDULOV, 1931). In culture both varieties have comparatively short leaves, but the dune race is much

smaller in size (stems and spikes abt. 30 cm., in var. *typica* abt. 50 cm.). Four samples of diploids were studied, viz. two from Ho and Skallingen in West Jutland (var. *intermedia*) and two from the Botanical Gardens in Helsingfors and Kaunas (var. *typica*).

Material of *K. glauca* from the Botanical Garden in Königsberg exhibited deviations in respect of morphology, earliness and chromosome number. The plants were higher (stems and spikes abt. 65 cm.), the leaves longer and not so rigid as in the rest. They were furthermore 7—10 days earlier and the cytological examination showed that they were tetraploid ($2n = 28$). Only one pair of chromosomes carried trabants, and in the diploids no trabants were observed.

The material of *K. pyramidata* var. *danica* was collected on a dry sunny slope at Frederikssund (North Zealand). 84 chromosomes were counted. It is the highest number known in the genus. In a related species which was called »*K. cristata*» AVDULOV (1931) found 70 chromosomes of almost the same shape and size as those of *K. pyramidata* var. *danica*. The plant counted by AVDULOV may belong to one of the 15 species of the *cristata* section which occur in the U.S.S.R. Another related type from the Botanical Garden in Delft (Holland) was cultivated in Copenhagen. The plant had been called *K. glauca*, but it hardly belongs to this species, the leaves being less bluish green and the leaf-margins having very scattered hairs of the same size as those of *K. pyramidata*. It may be a plant of the »*cristatae verae*», but its determination must be put off for the present. In some respects it resembles the tetraploid *K. glauca*, having up to 70 cm. long stems and spikes. Its chromosome number was determined as $2n = 42$. In some of the somatic metaphases two chromosomes carrying trabants were observed. Thus, in the *K. glauca* section the haploid numbers 7 and 14 occur and in the *K. cristata* section the numbers 21, 35 and 42.

Undoubtedly more countings and cultivations of species belonging to this genus are very desirable. Investigations of the West-European dune species *K. albescens* would be of special interest. This species also belongs to the »*cristatae verae*», but its variety *glabra* approaches *K. glauca* var. *intermedia* geographically, ecologically and in its habit.

The author would be very grateful to botanists and cytologists who would assist him in collecting seeds of wild-growing *Koeleriae* from Europe. The seed samples may be sent to the Plant Anatomical Laboratory of the University of Copenhagen.

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